The conservation ecology of Canterbury mudfish (*Neochanna burrowsius*)

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A pool on Dog Kennel Stream, South Canterbury in 1998
that contained an abundance of Canterbury mudfish fry.

'*It is a paradox of our times that a creature which can grow and breed in such a tiny scrap of habitat is so rare*' Eldon (1986, p. 19).

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Abstract

The Canterbury mudfish (*Neochanna burrowsius*; Galaxiidae) is a small, acutely threatened freshwater fish endemic to South Island, New Zealand, which has traits related to its historic occurrence in the wetlands of the Canterbury Plains. These wetlands have been largely transformed into productive agricultural land, with consequent fragmentation and local extinction of *N. burrowsius* populations. *N. burrowsius* now persists primarily in agricultural waterways across these increasingly drought-prone plains. I examined aspects of *N. burrowsius* habitat, inter-specific interactions, and reproduction of importance for their conservation. Field studies focused on four sites identified as important remnant *N. burrowsius* habitats. These were distributed across the latitudinal range of *N. burrowsius* and encompassed different hydrological and agricultural disturbance regimes, and fish communities.

Distributions of *N. burrowsius* within each site were patchy. Furthermore, the location of *N. burrowsius* aggregations within sites was temporally variable, and was influenced by changes in habitat quality and the presence of predatory fish. Predatory fish also affected *N. burrowsius* activity and abundance. At sites with intermittent flow that regularly dried up, successful strategies of drought survival were dependent on the size of *N. burrowsius*. Comparisons between populations indicated differences in size structure, individual growth, recruitment, deformities, disease, and parasitic infection. Experimental investigations highlighted the behavioural and physiological plasticity of *N. burrowsius*' responses to competition, environmental conditions during spawning, and the ability of embryos to tolerate hypoxia that likely aid survival in wetland habitats. Macrophytes were also revealed to be a key element in *N. burrowsius* habitat, with particular macrophyte species associated with different *N. burrowsius* life stages, and providing spawning substrate critical to the survival of eggs.

This research indicates that hydrological disturbance and the presence of predatory fish likely had a greater effect on population characteristics than the presence of competitors, geographical location, and direct agricultural disturbance. Thus, recognising these factors will be critical in the conservation of *N. burrowsius*.

Chapter 1.

General introduction to *Neochanna burrowsius*.

Freshwater fish stocks are declining worldwide (Moyle 1995; Warren et al. 2000); yet endemic fish species receive relatively little research or conservation attention (LeCren 1990; Maitland 1995; Johnston 1999). Often less is known about rare or threatened fish species, than those that are more common, or are valued as a human food resource (LeCren 1990). Consequently, the paucity of information available for many species is a barrier to determining appropriate conservation actions (Hicks & Reeves 1994). Ichthyologists have been implored to address this problem. Pister (1999) stated that it was the professional responsibility of researchers to participate in the preservation of freshwater fishes. Indeed, unless knowledge is improved it will be difficult to solve the increasing conflict between conserving fish species and the human need for land and water resources (May & Brown 2002). In New Zealand, this tension is pronounced in the lowland plains of the east coast, where extensive areas of wetland have been drained, and irrigation is now required to improve land productivity (McDowall 1998; Morgan et al. 2002).

Globally, and in New Zealand, the extent of lowland wetlands has contracted, largely due to anthropogenic disturbance (Wilson 1982; Lemly 1994; Cromarty & Scott 1996; Bourne 2000; Jenkins et al. 2003). New Zealand has lost an estimated 90 % of its wetlands in the last 150 years (Johnson & Gerbeaux 2004), yet wetland drainage still occurs legally, albeit at a much slower rate. Continued wetland loss is of concern as the structural complexity of wetlands and their unique hydrology, can provide important refugia for endemic fish, especially from introduced predatory fish (Chapman et al. 1996, 2002a). In New Zealand, wetland loss has directly threatened the persistence of species in the genus *Neochanna* (Galaxiidae; McDowall 1982, 1998; DoC 2003).

Neochanna contains six species, five of which are endemic to New Zealand. *Neochanna* occur throughout most of the low-lying areas of New Zealand in allopatric distributions. The focus of this thesis, Canterbury mudfish (*N. burrowsius*) occurs on the alluvial Canterbury Plains on the east coast of South Island, New Zealand.

Conservation and research

N. burrowsius was originally described by Phillipps (1926), from a sample sent to him by Mr A. Burrows, of West Oxford. A further specimen collected from Ashburton was reported on by Stokell (1938). By this time, the Canterbury Plains had been transformed from vast wetlands into productive farmland. Although much of the evidence is anecdotal, *N. burrowsius* is likely to have been prevalent in these former wetlands (McDowall 1998). By the late 1960s there was serious concern for *N. burrowsius*' persistence, and thus Skrzynski (1968) published all known information on the species, entitling his paper 'a vanishing species.' Skrzynski (1968, p. 695) concluded that 'it seems unlikely that Canterbury mudfish will continue to survive on the Canterbury Plains with the land continually being modified.' Subsequent authors have emphasised this point, e.g. Cadwallader (1975a), Eldon (1979a, 1993), and McDowall (1998). Indeed, local extinction has been on-going; Skrzynski (1968) listed nine locations where *N. burrowsius* specimens had been collected, but re-confirmed their occurrence in only four. Cadwallader (1973) found *N. burrowsius* in 13 locations, but failed to locate any in a seven previously occupied habitats. Whereas, Eldon (1979a) located 20 *N. burrowsius* populations, however also noted one or two local extinctions. Several further populations are now also thought extinct (Eldon 1993).

G. A. (Tony) Eldon started study on *N. burrowsius* in 1975 and published a series of papers on their habitat and inter-specific relationships (Eldon 1979a); diet (Eldon 1979b); and breeding, growth, and aestivation (Eldon 1979c); and their response to a severe drought (Eldon et al. 1978). During the 1980s, research attention was directed to the physiological adaptations of *N. burrowsius* (Meredith 1981, 1985; Meredith et al. 1982; Wells et al. 1984). Efforts were also being made to establish new populations of *N. burrowsius* (Eldon 1983, 1986, 1988a, b, 1989; Eldon & Field-Dodgson 1983). The 1990s saw an increased effort to manage the species with conservation guidelines being written (Eldon 1993). *N. burrowsius* was noted as a taonga (treasured) species in the Ngäi Tahu Deed of settlement, 1997, clause 12.14 (Te Rünanga o Ngäi Tahu & Her Majesty the Queen 1997). Moreover, a protective covenant was placed on an area containing *N. burrowsius* in Dog Kennel Stream, South Canterbury (Gray 2000). Research into the genetic population structure of *N. burrowsius* found low levels of genetic diversity, further highlighting its vulnerability (Davey et al. 2003). Currently, *N. burrowsius* is classified by the Department of Conservation as an acutely threatened species that is Nationally Endangered (Hitchmough 2002), and included in a threatened species recovery plan (DoC 2003). Recently, a report detailing the captive management of *Neochanna* species was published (O'Brien & Dunn 2005).

Biological background

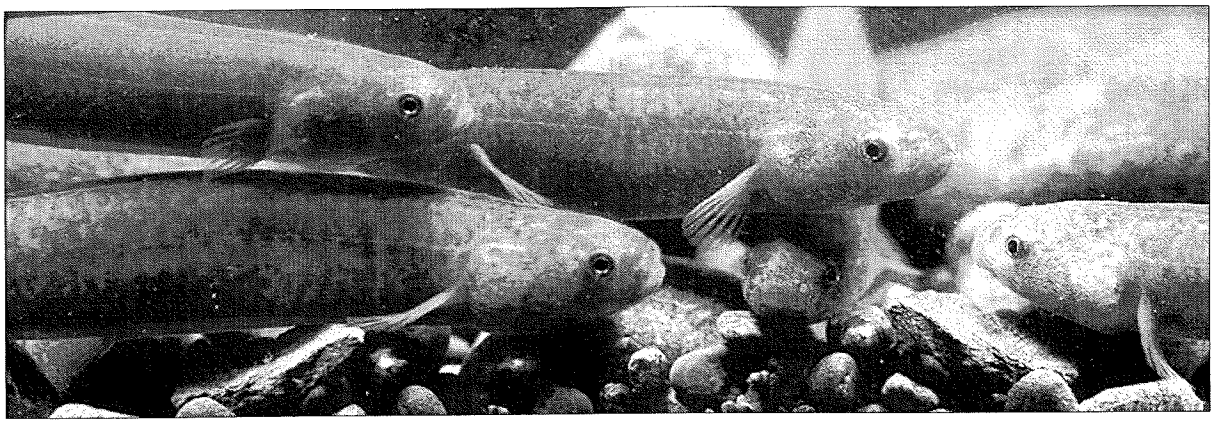

Figure 1.1. Canterbury mudfish (*N. burrowsius*).

N. burrowsius are small (< 160 mm), scaleless, cylindrical fish, with anguilliform locomotion, laterally placed pectoral fins, reduced pelvic fins, and a fleshy caudal peduncle (Figure 1.1; McDowall 1990). It is thus considered morphologically adapted to cluttered wetland habitats, with an ability to move through dense vegetation (McDowall 1990). *N. burrowsius*, like many other Galaxiidae, has an amphibious nature (Meredith 1985). Placed in hypoxic water *N. burrowsius* will gulp air and readily leave the water if conditions exceed their physiological tolerances (Meredith 1981). *N. burrowsius* have high rates of cutaneous respiration and a low resting metabolic rate, resulting in a tolerance of emersed conditions. Thus, they can survive at least one month out of water in laboratory conditions (Meredith 1985). These traits have allowed *N. burrowsius* populations to persist in spring- or seepage-fed wetland pools and streams with fluctuating water levels and flow. In the wild when habitats have dried up, *N. burrowsius* have been found in holes within soil banks, among tree roots, under logs and vegetation, and in subterranean cavities (Eldon et al. 1978; Eldon 1979a, c).

N. burrowsius are a non-territorial, non-migratory fish. During early spring females scatter up to several thousand eggs amongst vegetation at the water surface (Eldon 1979c). Fry and juvenile fish are pelagic and diurnal, whereas adults are nocturnal, benthic, and cryptic, thus they are rarely seen by the casual observer (Eldon 1979c). *N. burrowsius* have traits common in species found in disturbed environments, such as high fecundity, early sexual maturity (0+; Eldon 1979c), and a tendency to disperse and colonise new habitat (Eldon et al. 1978). By virtue of these traits, *N. burrowsius* has persisted despite its wetland habitat and the hydrology of the Canterbury Plains being radically and irreversibly changed.

The Canterbury Plains

The Canterbury Plains were formed by the deposition of outwash gravels transported from the Southern Alps by large braided rivers, which shaped extensive alluvial fans. These gravels created confined aquifers as the materials deposited during glacial periods were overlain by oceanic deposits during interglacial marine transgressions when sea levels were higher (Fitzharris et al. 1992). These processes have led to the development of artesian springs and aquifers which occur across the plains. Four large braided rivers bisect the plains, being fed by alpine lake systems close to the main divide (Figure 1.2). Thus, these river systems often receive orographic rainfall that does not reach the easterly plains, and have high base flows. Annual rainfall on the east coast is often less than 700 mm, resulting in a moisture deficit during both summer and autumn (Fitzharris et al. 1992). This low rainfall meant that between the large alpine river systems, smaller rivers with foothill catchments rarely had the flow to maintain a channel to the sea, continuously. Connection to the sea was also impeded by long stretches of gravel bars and dunes formed as wave action piled alluvial gravels against the shore (Fitzharris et al. 1992). Furthermore, the east coast of the South Island is gradually tilting due to movement of tectonic plates, resulting in the formation of high cliffs along sections of the coast (McDowall 1998). These factors acted to retain what little rainfall reached the plains in coastal wetland and lagoon systems that extended the length of the Canterbury Plains. In addition, depressions between the alluvial fans of the main rivers collected water, forming inland wetlands that were drained by ephemeral watercourses such as the Selwyn River, which flows into Lake Ellesmere; and the Hinds River (Davis 1984; Fitzharris et al. 1992). Jobberns (1927, p. 93) commented on these inter-fan depressions that 'being by structure adapted to the accumulation of ground water and the surface silts from the higher surrounding land, these localities comprise some of the richer lands of the [Canterbury] Province, and some have had to be reclaimed from an original swampy condition by artificial drainage.' It was within these extensive inland depressions and coastal wetlands that *N. burrowsius* was likely to have been common and some populations still persist in remnants of such habitat (Figure 1.2).

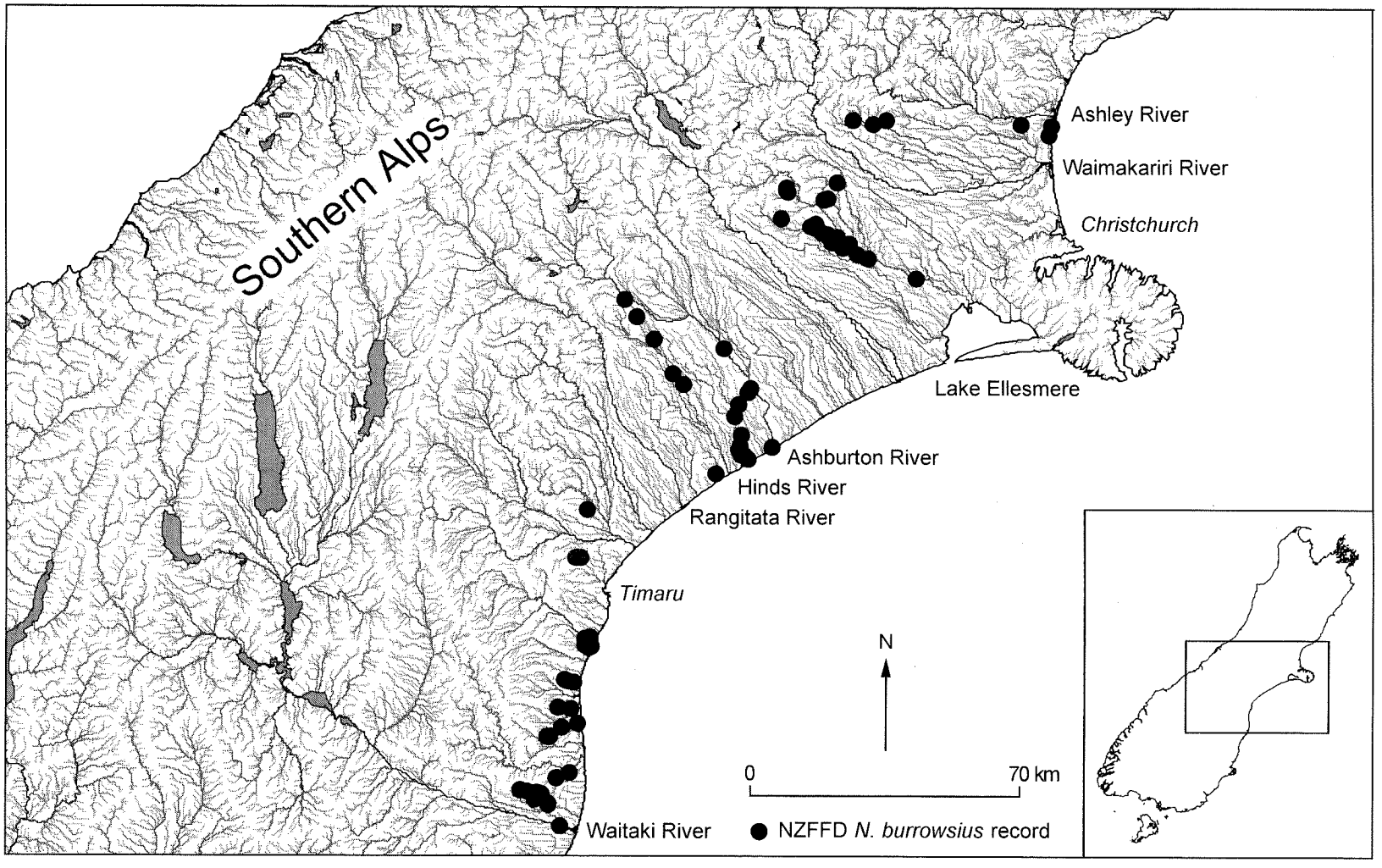

Figure 1.2. Drainage network of the South Island, New Zealand overlaid with locations of recorded *N. burrowsius* populations. The Canterbury Plains is apparent from predominantly parallel waterways. Records were obtained from the New Zealand Freshwater Fish Database (NZFFD; McDowall & Richardson 1983) downloaded on the 16 May 2005 and mapped using the Freshwater Fish Database Assistant 5.1 (Jowett 1998). Locations shown include many known to have gone locally extinct, but do not include translocated populations. Inset shows complete outline of South Island.

N. burrowsius habitat fragmentation

To understand the current plight of *N. burrowsius*, it is important to consider the historical changes to its habitat. Before human arrival, much of the Canterbury Plains were covered with podocarp forest dominated by kahikatea (*Dacrycarpus dacrydioides*). These lowland swamp forests were removed by Polynesian fires and were replaced by scrub, tussock and wetland vegetation (McDowall 1998). Early settlers described much of the expansive plains as 'interminably monotonous' 'as far as the eye could see' covered with 'impenetrable bogs' (McDowall 1998). However, the flat expanse of the Canterbury Plains proved irresistible to early settlers, who invested considerable effort into transforming the landscape. Conversion was surprisingly rapid, for example much of the area between the Ashburton and Rangitata rivers was changed from a 'dismal swamp' to highly productive farmland in 15 years by a Mr John Grigg of Longbeach (McDowall 1998). This was achieved by digging extensive channels and laying nearly 250 km of tiled field-drains (McDowall 1998). However, the greatest change was the channelisation of the Hinds River so that it drained directly to the sea, where once it dispersed into a vast wetland near the coast (McDowall 1998). It is not known if Mr Grigg found *N. burrowsius* while draining this wetland, however they have persisted there. This area provides a graphic example of the extent of *N. burrowsius* habitat fragmentation (Figure 1.3). As McDowall (1998, p. 37) commented *N. burrowsius*' 'latitudinal range is still about the same as it originally was, but it is now restricted to pathetic residual fragments of wetland that survive, scattered across the often dry and parched Canterbury Plains.' This fragmentation may have occurred faster than existing stochastic processes of extinction, meaning that an 'extinction debt' is likely to be present. The concept of extinction debt relates to the temporal lag between habitat fragmentation and subsequent local extinction due to stochastic processes (Hanski & Ovaskainen 2002). Fragmentation can also lead to reduced genetic diversity with consequences, such as reduced fitness and accumulation of deleterious mutations (Wang et al. 2002; Gaggiotti 2003). This situation is compounded in *N. burrowsius* because they have low intra- and inter-population genetic diversity, with some populations having no detectable nucleotide diversity (Davey et al. 2003). Although the loss of genetic diversity does not necessarily indicate an immediate threat, it may be detrimental to long term persistence. Importantly, a population may lose the capacity to adapt to novel selection pressures as the ability to respond to changing conditions is proportional to the diversity of underlying genetic traits (Gaggiotti 2003). Thus, continued fragmentation and habitat change are likely to be detrimental to *N. burrowsius*' long term chances of persistence.

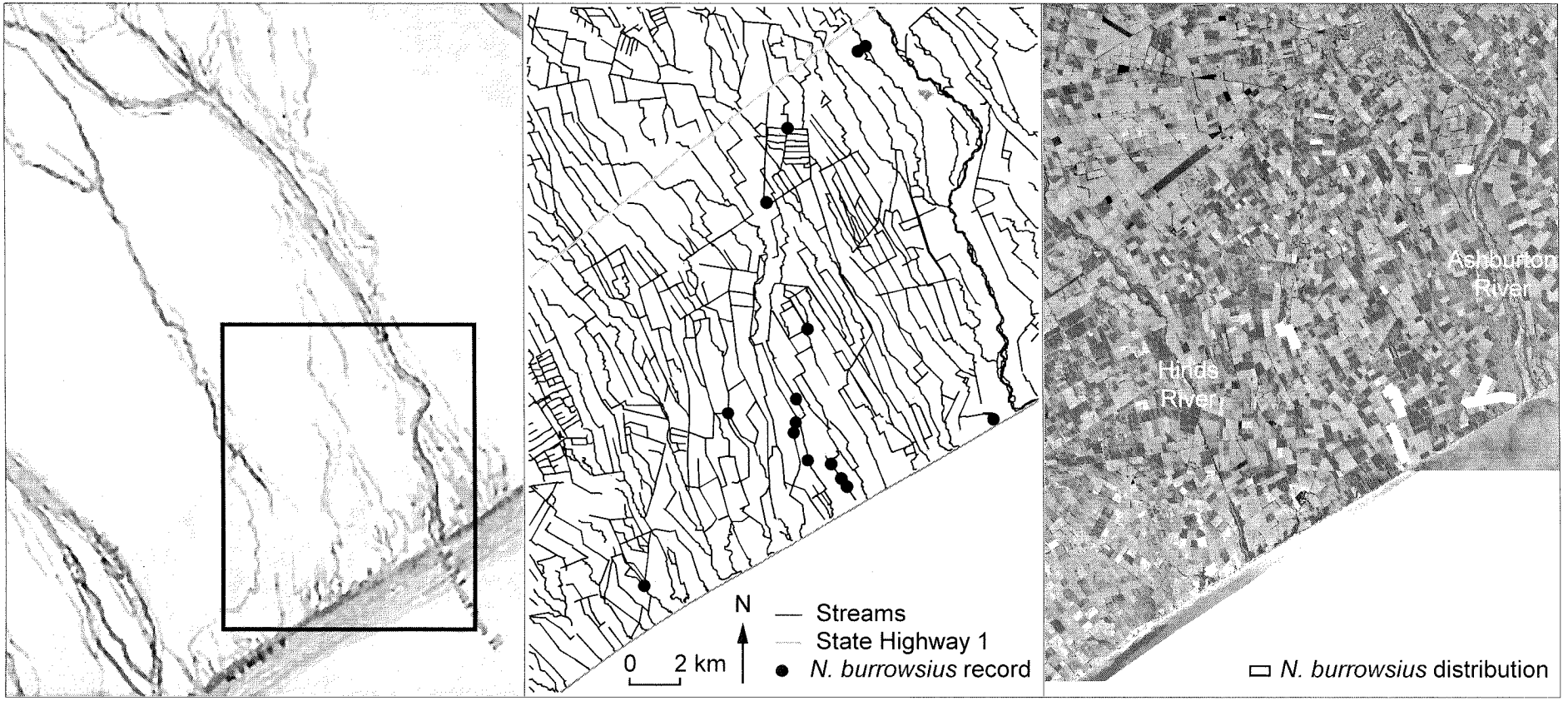

Figure 1.3. A case study of habitat fragmentation that has occurred in the Longbeach area between the Ashburton and Hinds rivers, south of State Highway 1. Left = modified 1800s Black Map, Middle = New Zealand Freshwater Fish Database records of *N. burrowsius* as at 1 November 2004 overlaid with the stream network of the area, Right = distribution of *N. burrowsius* as at 15 November 2004 overlaid with an orthograph of the area (Environment Canterbury unpublished data). Note the presently channelised lower section of the Hinds River which now discharges to the South Pacific Ocean.

Ideal habitat

Skrzynski (1968, p. 694) concluded that 'it now seems impossible to determine the original habitat' of *N. burrowsius*. This fact has been widely recognised, however the nature of *N. burrowsius*' original habitat has been discussed, frequently (Eldon 1979a; Meredith 1985; McDowall 1990). Many of the first specimens were collected from habitats after they had dried up (Phillipps 1926), thus, Skrzynski (1968, p. 694) 'assumed that it occupied isolated waters which dried periodically.' However, Eldon (1979a) debated this point and emphasised the similarities between *N. burrowsius* habitat and tropical hypoxic wetlands where air-breathing fish also occur. Moreover, Meredith (1981, 1985) showed that *N. burrowsius* exhibited physiological and behavioural adaptations most likely to have evolved in swamp forest pools where hypoxia was frequent, and that it showed little adaptation to habitats that completely desiccated. Yet, *N. burrowsius* occur frequently in seasonally harsh environments where low dissolved oxygen and the near absence of water during summer exclude many other fish species. Despite this, *N. burrowsius* is considered a 'clean water species' requiring generally high water quality (McDowall 1998).

N. burrowsius habitat is predominantly spring fed wetlands, with water sourced either from hill seepage, or groundwater, often supplied by an adjacent river. Due to the proximity of hills and rivers these sites frequently experience scouring floods capable of creating pool habitat (Eldon 1979a; personal observation). Eldon (1979a) described ideal *N. burrowsius* habitat as still or very slowly flowing, meandering, 'swampy streams with deep pools that retained water for long periods after flow had ceased.' Similarly, Skrzynski (1968) described suitable habitat as 'a small creek with very little flow (about 0.1 cusec), which had deep pools with flax and rush cover, and a mud bottom.' However, *N. burrowsius* has been found in a variety of habitats, including seepage streams, spring streams, dams, farm ponds, scour holes, stockwater races and possibly, subterranean habitat (Eldon 1979a). The diverse range of habitats in which *N. burrowsius* is found, may in part be a consequence of the considerable modification of its habitat, whereby *N. burrowsius* are forced to occupy whatever habitat remains. Indeed, in many areas *N. burrowsius* now occurs only in the channels used to drain its former wetland habitat. Thus, although *N. burrowsius* may be a wetland specialist by virtue of its morphological and physiological adaptations (Meredith 1985; McDowall 1997a), often the habitat that it now occupies cannot be described as wetland.

Current threats

In less than 40 years most wetlands on the Canterbury Plains were converted to farmland (McDowall 1998). Today, there is little visible evidence of these wetlands and the hydrology of the plains has altered, leaving many formerly waterlogged areas experiencing seasonal soil moisture deficit and resulting in an increase in both the frequency and severity of drought disturbance (Sagar & Jellyman 1985; McDowall 1998). This has lead to many fish strandings (e.g. Hicks 1982; Sagar 1985; Jellyman 1989; Main & Meredith 1999). As McDowall (1998, p. 39) concluded, 'the ultimate irony is that the formerly extensive wetlands of Canterbury are now highly productive farmland whose full potential can be realised only by irrigation.' Unfortunately, *N. burrowsius* is threatened by increased water abstraction for irrigation as the spring-fed habitats that it occupies are most sensitive to reductions in groundwater levels (Morgan et al. 2002). However, the influence of water abstraction on larger river systems is often given greater consideration, with water resources being balanced between agricultural and river systems, rather than small waterways. This has generated a new threat as *N. burrowsius* often occur in small stock water race systems. It has been estimated that the efficiency of these stock water races, i.e. the percentage of the abstracted water actually consumed by stock; can be as low as 3 % (Morgan et al. 2002). A strong case has been put forward to remove or pipe these watercourses to ease pressure on the in-stream values of larger rivers and free up water allocation for other uses (Morgan et al. 2002). Thus, the increasing human need for water is continuing to threaten *N. burrowsius* populations, both indirectly via water abstraction lowering groundwater levels and directly as agricultural water networks are piped to increase water use efficiency.

Several other aspects of waterway management are also likely to threaten *N. burrowsius*. Any activity, such as drain maintenance and stock grazing, that removes in-stream vegetation also removes cover and spawning substrate required by *N. burrowsius* (Eldon 1979c). Stock access can also cause compaction of substratum, and break down banks, which may reduce refuge for *N. burrowsius* (Eldon 1993). Another issue is that *N. burrowsius* is considered to have low competitive ability because it occurs on its own in the majority of recorded sites (Eldon 1979a; Meredith 1985). Changes in hydrologic regime caused by irrigation, and channelisation or realignment of remaining habitat, may affect *N. burrowsius* by improving habitat for other fish species. Thus, actions that increase baseflow, velocity, connectedness, and remove dispersal barriers to predatory shortfin eel (*Anguilla australis*) may be detrimental to *N. burrowsius* by increasing competition and predation (Eldon 1979a).

Thesis outline

My general aim in this thesis was to examine the conservation ecology of *N. burrowsius*; to explore methods of assessing habitat quality and population persistence; and to identify factors limiting population growth. This thesis is written as a series of manuscript style chapters with one collated reference list and a final summary chapter. The thesis has been separated into four sections covering a variety of topics considered important to the conservation of *N. burrowsius*. The initial section includes three chapters on aspects of survey methodology. These chapters cover the capture methods used for *N. burrowsius*, and examine methods of monitoring population decline, and habitat suitability. The second section comprises two chapters assessing the dynamics and health of *N. burrowsius* populations occurring in remnant habitats spread across its distributional range. These chapters compare a number of measures used to assess individuals and their populations, including, size, density, biomass, average condition and growth, as well as, ecto-parasite and infection loadings. The third section consists of chapters examining two common limiting factors for *N. burrowsius* populations, i.e. drought disturbance and inter-specific interactions. The final section contains three chapters examining reproductive characteristics. These chapters evaluate the importance of environmental conditions on *N. burrowsius* spawning, and examine survival and development through the early life stages of *N. burrowsius* as eggs, larvae and fry.

Study sites

Eldon (1993, p. 7) stated that 'four locations are considered to be very important in terms of supporting mudfish populations.' These four populations are the central focus of studies in this thesis and were briefly described by Eldon (1993). Unfortunately, in-depth field investigations involving conservation species are inevitably restricted due to the rarity of individuals or populations. Indeed, the paucity of sufficiently large populations hampered earlier field studies of *N. burrowsius*, e.g. Cadwallader (1973); with Skrzynski (1968, p. 688) commenting that 'no established population, which could be studied in detail, has been found.' Although I visited and sampled numerous remnants of *N. burrowsius* habitat, data from only the four important sites described by Eldon (1993), were examined in detail in this thesis (Figure 1.4; Table 1.1). These sites contained persistent and sizeable populations compared with others, and represented a wide variety of habitats, thus are assumed to be representative of *N. burrowsius*' remnant habitat and its ecological situation, allowing valid generalisations to be made.

Table 1.1. Location of the four study sites in Canterbury.

Site	Latitude	Longitude	Altitude (m)	Distance inland (km)
Te Roto Repo o Tawera	43.29	172.08	310	75
Hororata Spring	43.59	172.04	140	60
St Andrews Drain	44.52	171.20	5	1
Dog Kennel Stream	44.88	171.03	100	16

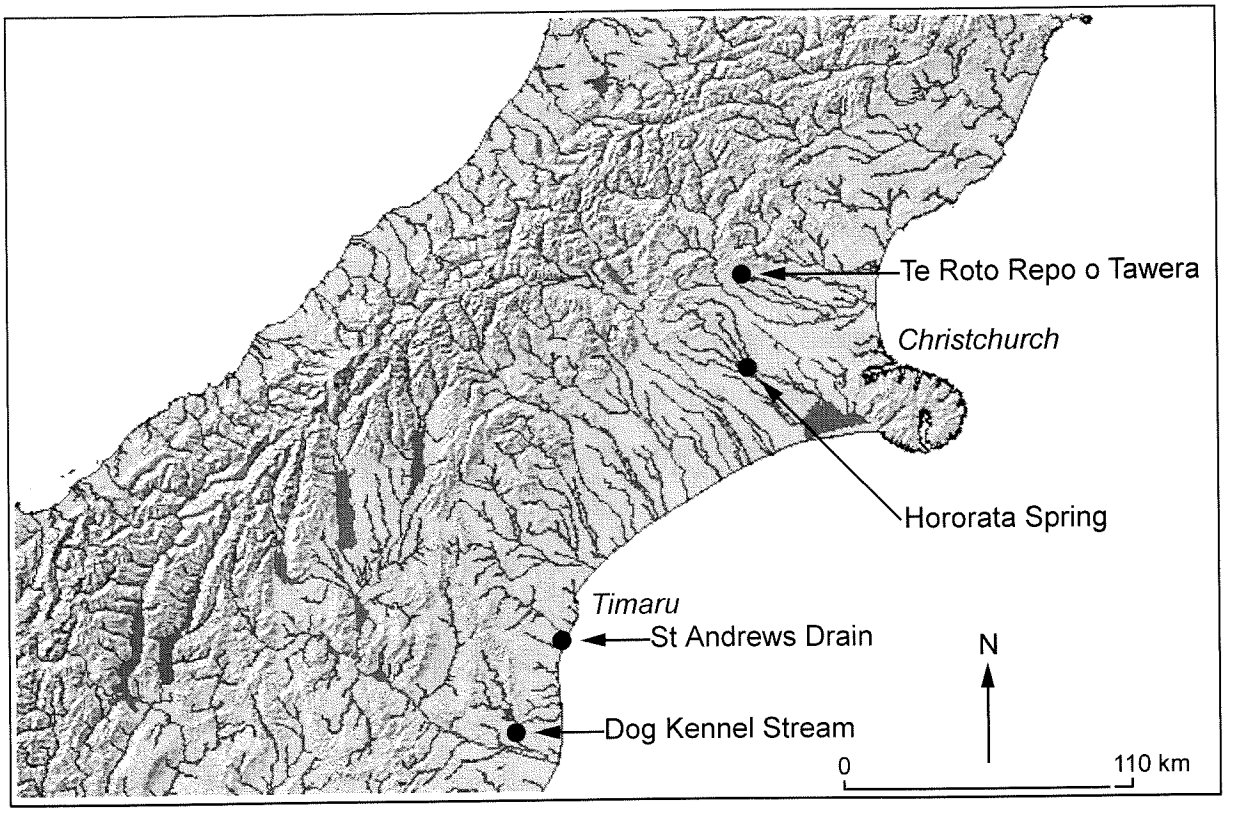

Figure 1.4. Location of *N. burrowsius* studies sites across the Canterbury Plains. Modified from NIWA (2004).

General site characteristics

The four study sites examined in my thesis were located across the distributional range of *N. burrowsius* (Figure 1.4, Table 1.1). All four sites were isolated or seasonally disconnected from the South Pacific Ocean. The intensity of agricultural land-use and level of stock access differed amongst sites. However, in all cases, sites were less intensively managed than surrounding farmland. The fish community in sites was depauperate, with no more than three species ever being found, i.e. *N. burrowsius*, upland bully (*Gobiomorphus breviceps*), and *A. australis*.

Mitochondrial DNA analysis of the population structure of *N. burrowsius* identified two evolutionary significant units (ESUs): Mid Canterbury and South Canterbury (Davey et al. 2003). In my study, both ESUs were represented by two populations. The four sites can also be ranked along a continuum of hydrological and morphological characteristics, with sites ranging from perennial to intermittent, and from streams to interlinked pools (Figure 1.5). The sites are also representative of the ecological and agricultural situation *N. burrowsius* commonly occupies. Two sites contained *A. australis*, and two sites contained *G. breviceps* populations; two sites perennially flowed and two sites flowed intermittently, and two sites had much lower levels of agricultural pressure than the other two (Table 1.2).

The various factors, i.e. ESU, hydrological regime, presence of other fish species, and the level of agricultural influence, are likely to have different strengths, and *N. burrowsius* is likely to respond to them differently. Thus, by comparing pairs of sites (as in Table 1.2), in relation to population responses, it may be possible to identify factors that are likely to be structuring or limiting *N. burrowsius* populations. However, some abiotic and biotic characteristics were confounded (Table 1.2). I know of no natural site that contains both *N. burrowsius* and *A. australis* that does not also experience intermittent flow and regular desiccation.

Table 1.2. Study sites arranged to illustrate the similarities between pairs of sites, in terms of evolutionary significant unit (ESU), hydrological regime, presence of other fish species, and the level of agricultural influence. Sites in bold had low levels of agricultural influence, whereas underlined sites had high levels of agricultural disturbance.

	Perennial flow *A. australis* absent	Intermittent flow *A. australis* present
Mid Canterbury ESU *G. breviceps* present	**Te Roto Repo o Tawera**	Hororata Spring
South Canterbury ESU *G. breviceps* absent	St Andrews Drain	**Dog Kennel Stream**

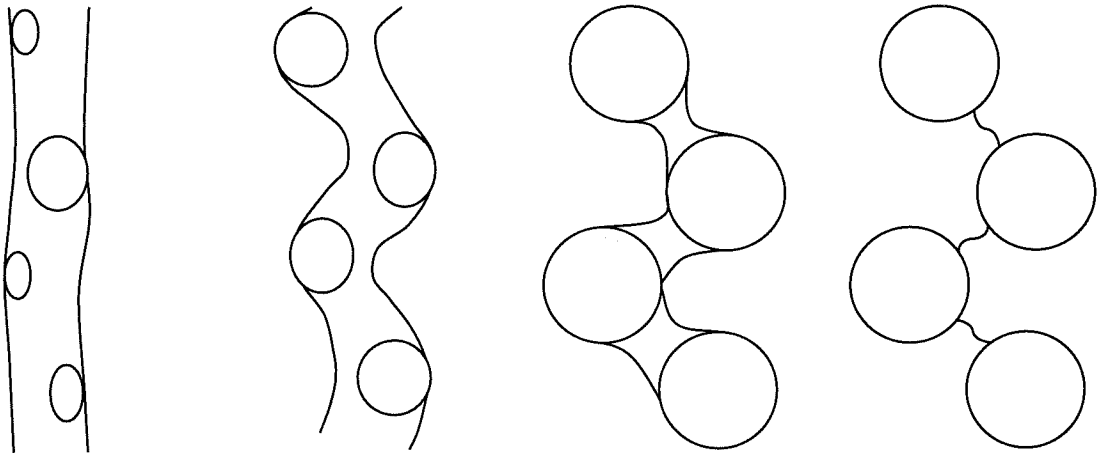

Figure 1.5. Generalised study site morphology. Circles represent pool areas, and lines stream runs. Sites can be placed, left to right, along a continuum from a channelised stream with little pool area to a series of pools connected by small intermittent runs. The sites on the left had perennial flow and those on the right had intermittent flow and dried up, to some extent each summer/autumn.

Te Roto Repo o Tawera

General landscape

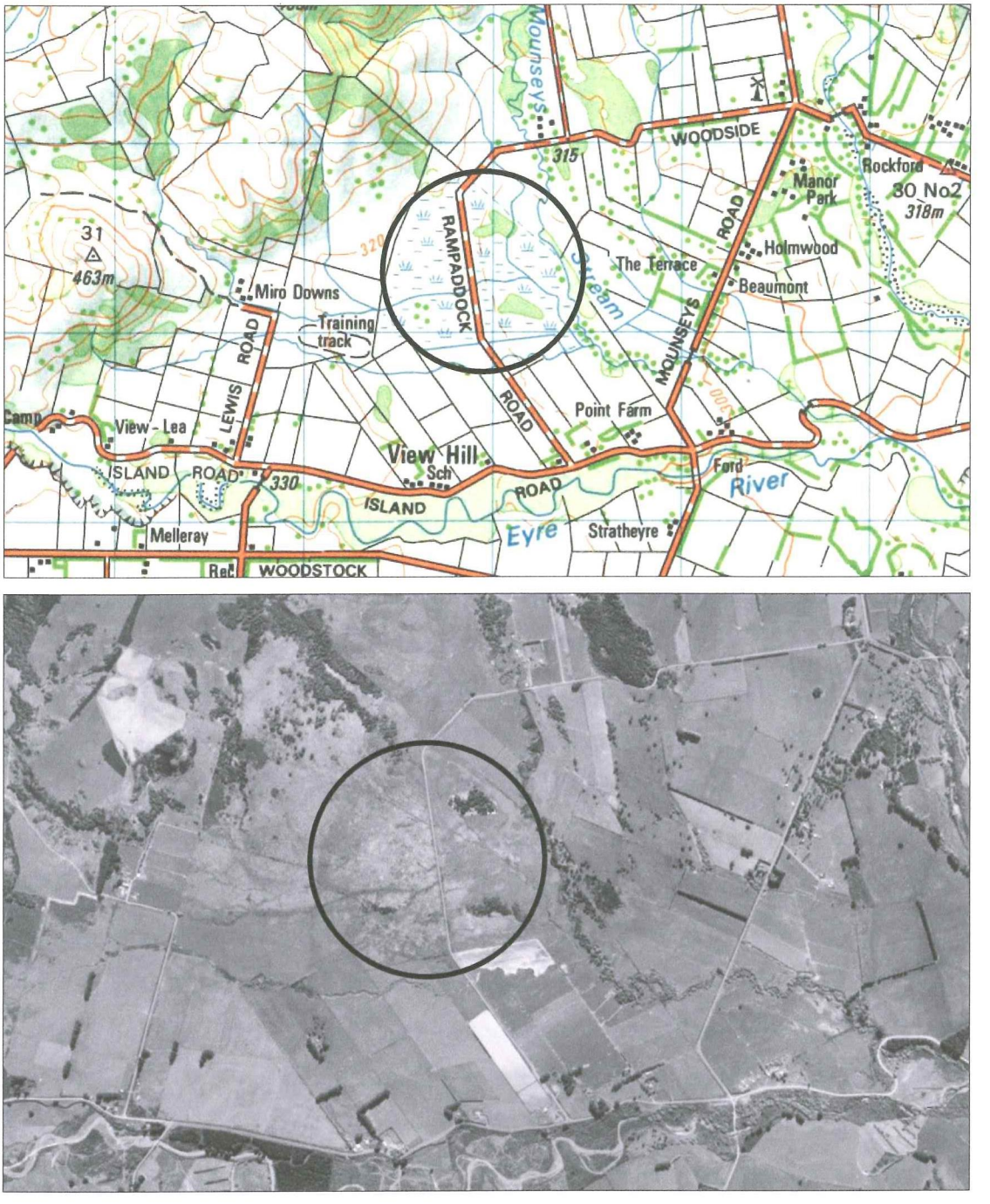

Figure 1.6. Te Roto Repo o Tawera, NZMS 260 L35 Waimakariri (2435000E, 5768300N) Topographical map (top) and orthograph (bottom). The main study site is within the circles. Grid is 1 km^2 in topographical map; north is at the top of the page in both views.

Te Roto Repo o Tawera is a 175 ha block of largely unmodified land, in West Oxford, Mid Canterbury (Figure 1.6). The area contains a diverse range of vegetation types, including remnant kahikatea forest swamp, rush (*Juncus* spp.) dominated wetlands, and rare plant species, such as the native liane broom (*Carmichaelia kirkii*) and spider orchids (*Nematoceras* spp.). Te Roto Repo o Tawera is situated at the foothills of the Southern Alps, and it is from these hills that it receives seepage flows. The relatively high elevation and inland placement of this wetland means that it can receive snowfalls, and ice frequently forms on standing water in winter (Eldon 1993). The main waterway perennially flowing through the wetland has a meandering channel with a distinct pattern of deep (~ 0.5 m) pools separated by shallow cobbled riffles. At the lower edge of the wetland the waterway flows into the willow (*Salix* spp.) lined Mounsey's Stream (Figure 1.7), which joins the intermittently flowing Eyre River, itself eventually joining the alpine sourced Waimakariri River to the north of Christchurch City. Eldon (1993) also described the site under the name Mounsey's Stream Wetland.

Te Roto Repo o Tawera was the least agriculturally modified *N. burrowsius* site sampled and it contrasts with the surrounding land (Figure 1.7) as pasture improvement involving the removal of native vegetation, tillage, and seeding with introduced pasture grasses has not occurred. A herd of horses graze the site, though they are mainly confined to higher ground and tend to use discrete stream crossings. No mechanical clearance of the waterway has occurred; although in the past, invasive vegetation was removed using hand tools. Nonetheless, the wetland is surrounded by drained, intensively modified farmland with regularly maintained ditches; consequently the hydrology of the site has undoubtedly changed since pre-European settlement. Gillespie (1954, p. 34) described hydrological changes of the Oxford area, which he attributed to the loss of bank side vegetation after the establishment of deer. He recognised that this overhanging vegetation 'helped to hold the water and steady its flow into creeks.' Without this vegetation he described how floods that once took 3 days to travel to Oxford in the 1880s and 1890s would take just 24 hrs. He also described the progressive widening and alteration of substratum that accompanied changes in the hydrological regime. He stated that 'it was possible then to jump from bank to bank with ease across the upper reaches of the Eyre River' and that 'smaller streams were merely the width of a step, bordered by rushes and flax and ranker bog plants.' He observed that these small waterways had changed and that 'today most of the larger streams are wide and boulder strewn.' It is likely that the extent of suitable habitat for *N. burrowsius* in the areas has declined as water velocities increased in these waterways.

Habitats sampled within Te Roto Repo o Tawera

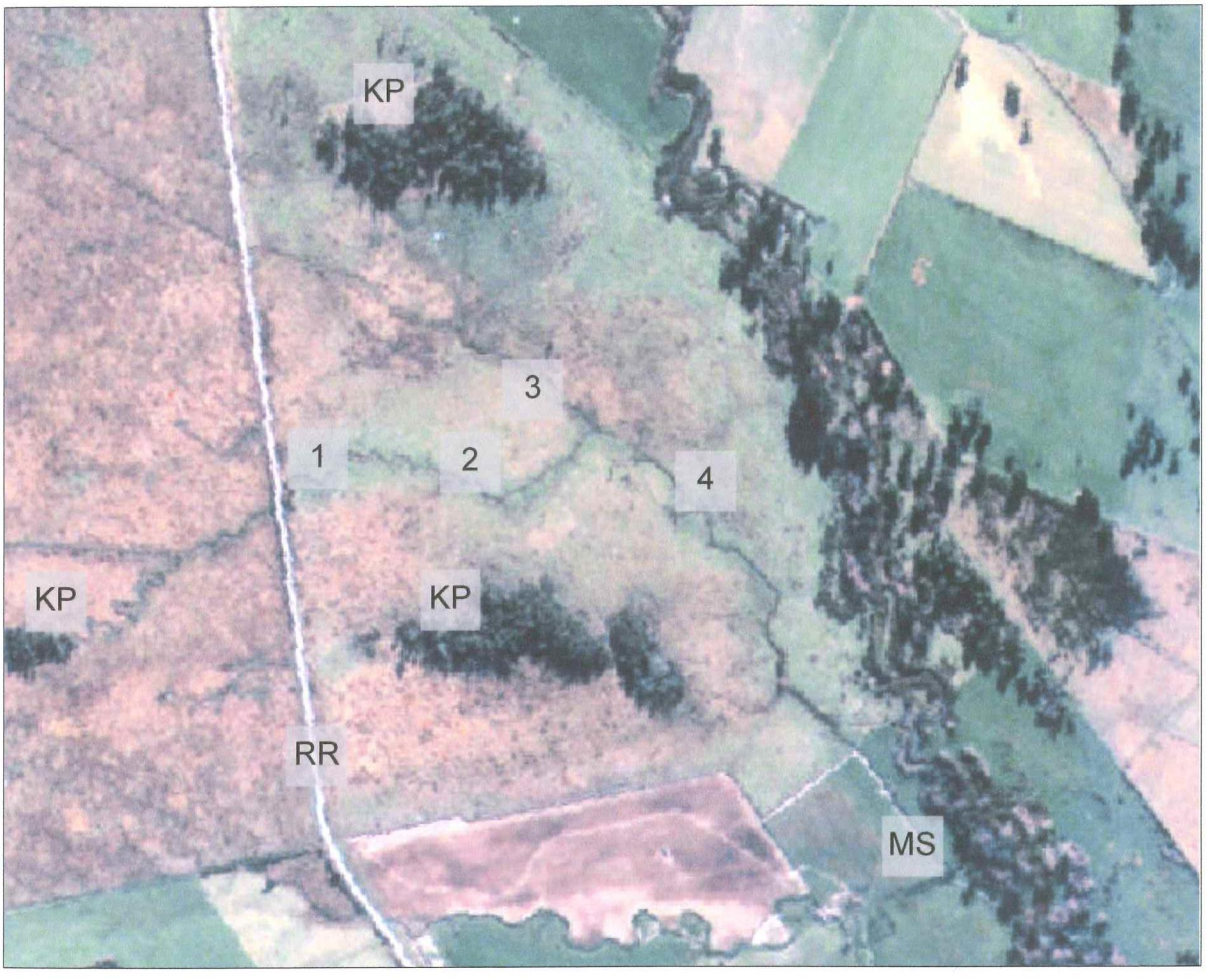

Figure 1.7. Approximate locations of habitats studied within Te Roto Repo o Tawera. Numbers indicate most intensively studied habitats, where 1 = Top, 2 = Middle, 3 = Tributary, 4 = Bottom. KP = Patch of Kahikatea, MS = the confluence with Mounsey's Stream, RR = Rampaddock Road.

Four habitats were repeatedly sampled (Figure 1.7), although initial sampling was conducted throughout the wetland. Three of these main habitats were spaced along the main watercourse and one on a tributary. These were named, Top, Middle, Bottom, and Tributary, respectively (Figure 1.8). Each reach was 100 m in stream length, representing a total area of 710 m^2. These habitats were also described and studied by Taylor & O'Brien (2000). The main stream channel has high sinuosity and well developed pool-run sections with short sections of cobbled riffle. The tributary contained permanent water in narrow deep pools shaded by *Blechnum* spp. ferns and small shrubs of *Coprosma* on one bank, they contained native and naturalised aquatic macrophytes. Riparian shrubs occur along the mainstem in areas providing shade, however these are dominated by gorse (*Ulex europesis*).

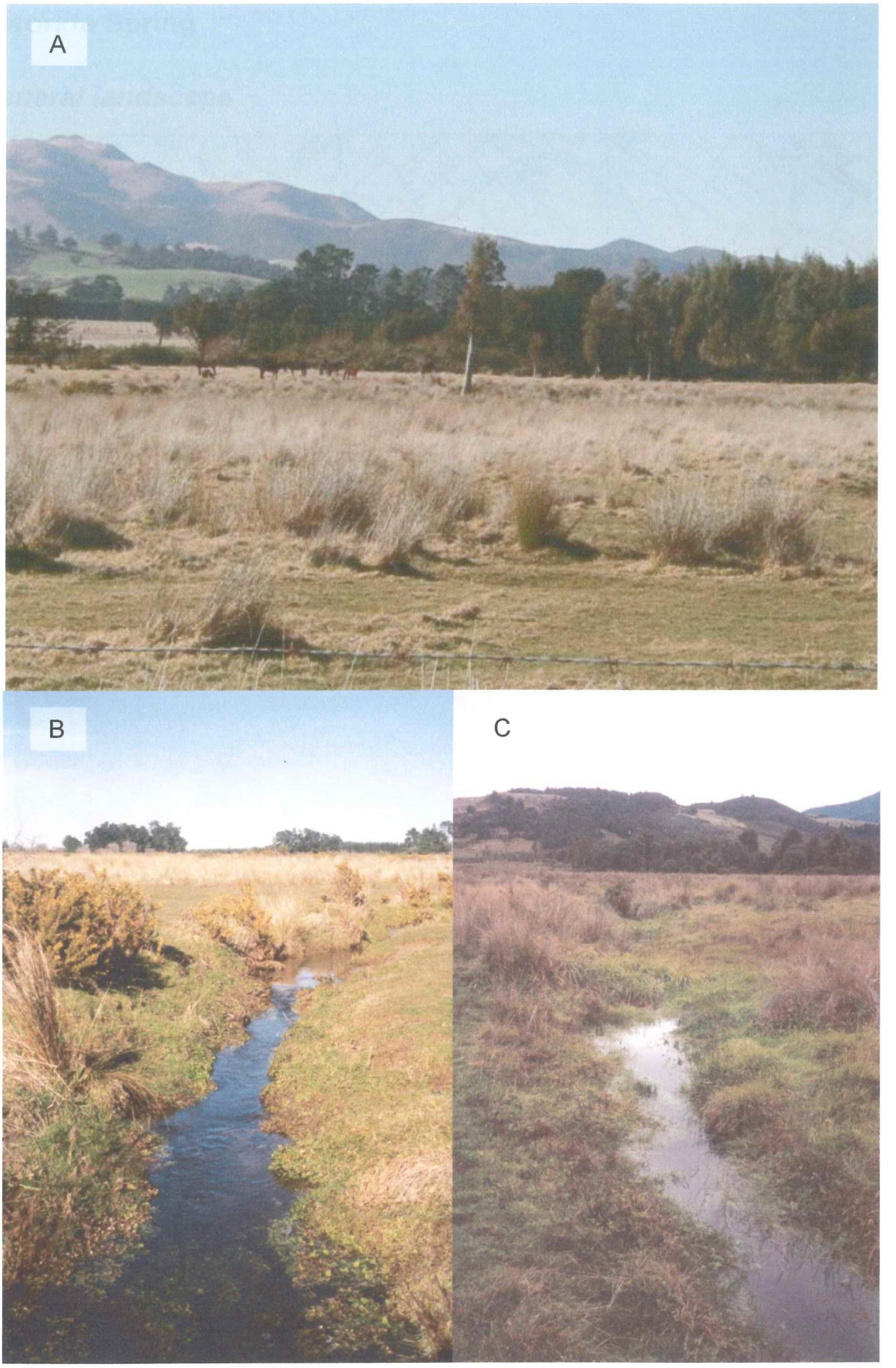

Figure 1.8. Unmodified pasture and remnant Kahikatea forest at Te Roto Repo o Tawera (A). Through this flows one main waterway (B) and a perennial tributary (C).

Hororata Spring

General landscape

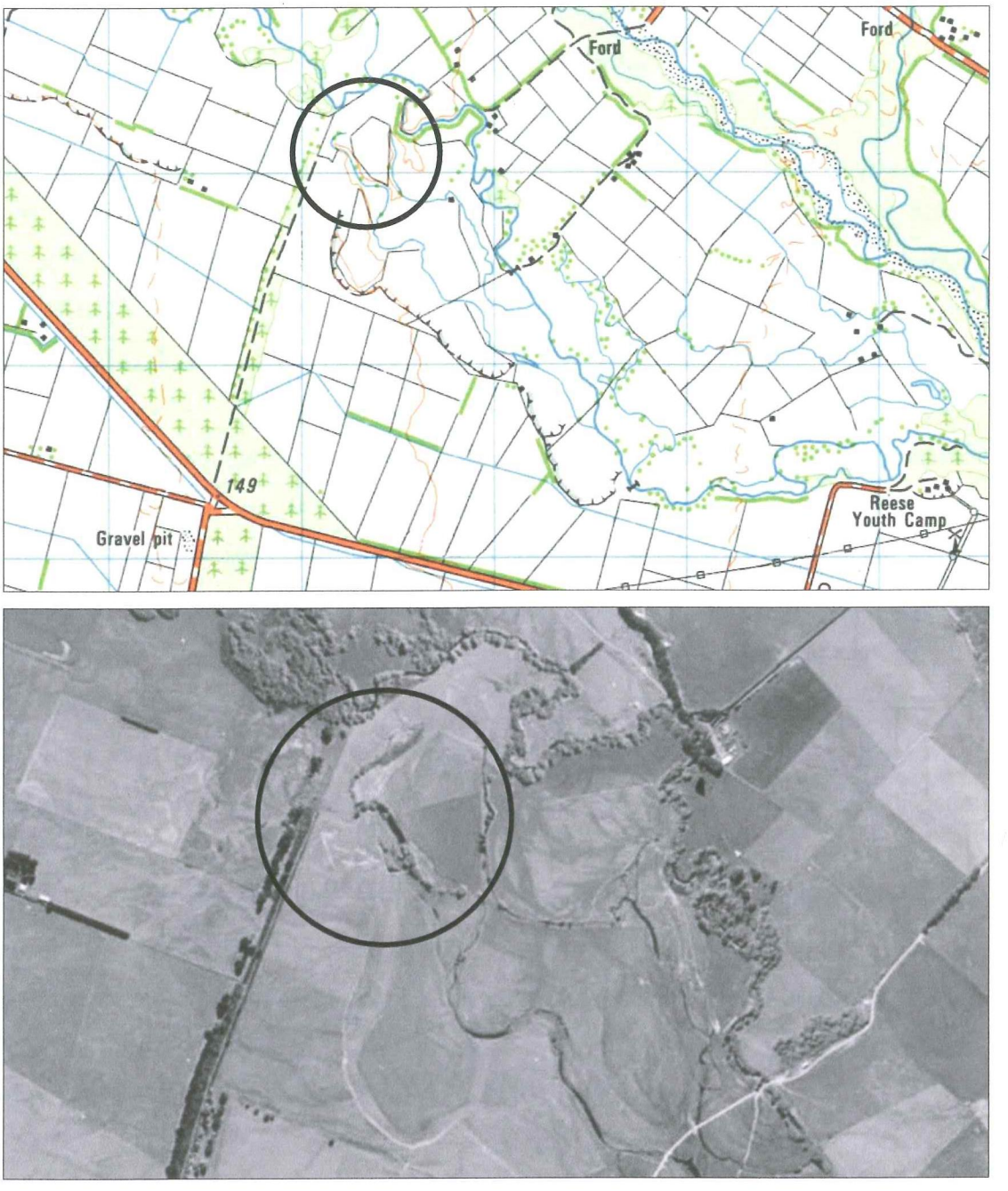

Figure 1.9. Hororata Spring NZMS 260 L36 Rakaia (2432200E, 5735100N) Topographical map (top) and orthograph (bottom). The main study site is within the circles. Grid is 1 km^2 in topographical map; north is at the top of the page in both views. The waterway adjacent to the site is the Hororata River; those to the far right in the top map are the Selwyn and Wainiwaniwa rivers.

Hororata Spring is a wetland complex of scour holes, spring-fed pools and slowly flowing stream runs, the morphology of which has not been modified by agriculture (Figure 1.9). Water emanates from groundwater springs that are linked to the underlying aquifer and the Hororata River. The Hororata is within the Selwyn River catchment, which itself flows into Lake Ellesmere. During the study period, Hororata Spring was the site that most frequently and intensely dried up, however it also experienced an extensive flood. During summer and autumn the site invariably contracted to a series of pools; however the system became connected and flowed during winter and spring. However, the site was never observed to completely dry up, with at least one deep pool always containing water, despite the nearby Hororata River completely drying up. It is thought that the Hororata River only began drying up in the 1930s; however this is now a regular occurrence (Davis 1984).

Intensive stock grazing was observed at Hororata Spring in years with very dry summers, and of the four sites this appeared to be the most influenced by agriculture. Grazing was predominately by sheep; however beef cattle were frequently present. Stock faeces were commonly seen in the waterway, either directly deposited there by cattle, or sheep manure washed into the waterway by surface flow. Further, a large, deep central pool was used for duck shooting and two maimai have been constructed. Scattered grain was often used to attract large numbers of ducks to the area prior and during the autumn shooting season. Large flocks of a wide variety of birds would also frequent the site during especially dry summers, suggesting that it may, at times, hold the only remaining water in the area. Despite the high density of ducks present, extensive beds of water milfoil (*Myriophyllum propinquum*) occurred in deeper areas of pools. Further, although aquatic and riparian vegetation was frequently grazed down by both ducks and stock, this tended to occur at discrete periods and vegetation quickly recovered. The importance of this site for the conservation of *N. burrowsius* was detailed by Eldon (1993), and O'Brien (2001).

Habitats sampled within Hororata Spring

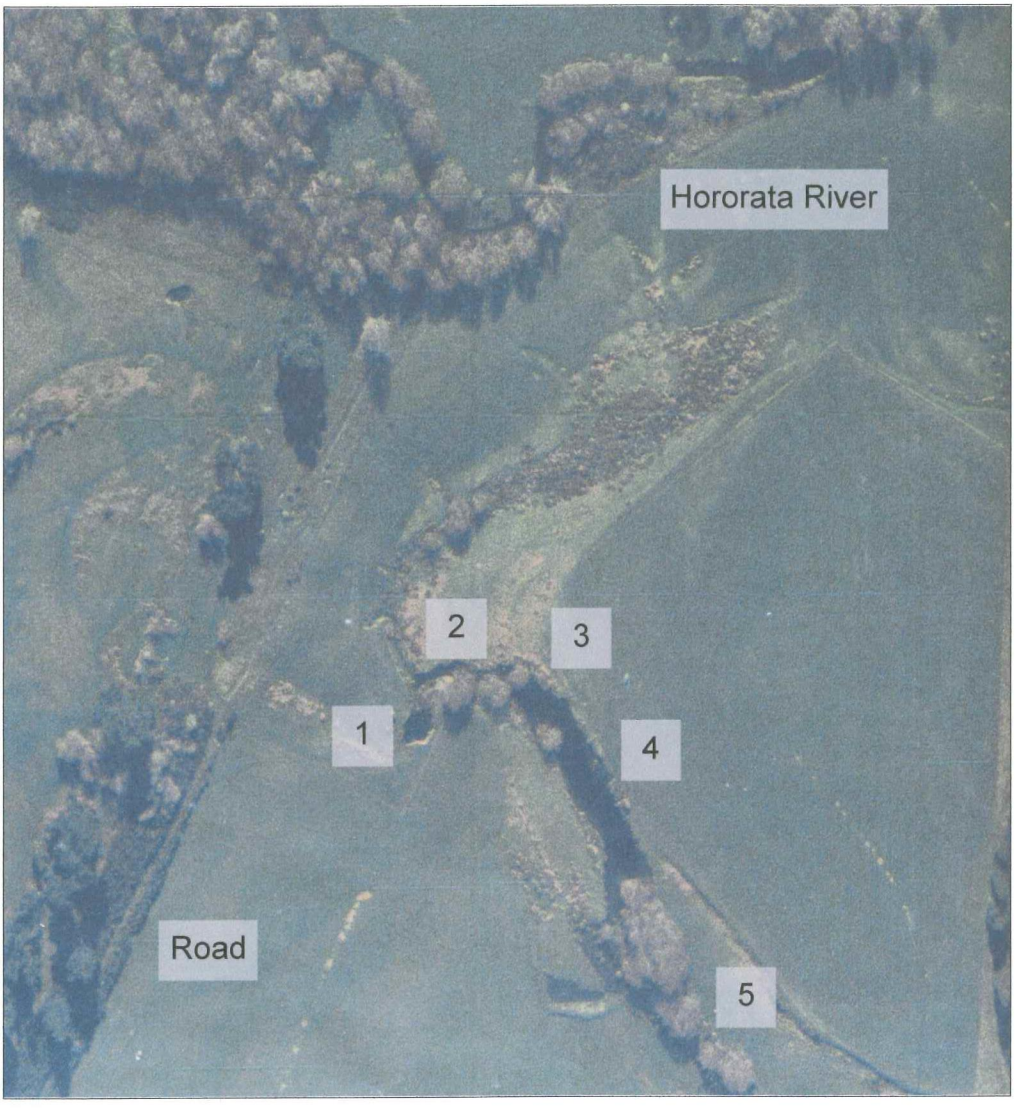

Figure 1.10. Approximate locations of habitats studied within Hororata Spring. Numbers indicate most intensively studied habitats, where 1 = Spring Pool, 2 = Confluence, 3 = Willow Pool, 4 = Duck Pond, 5 = Wetland. At the top of the picture is the Hororata River.

Five different habitats were identified (Figure 1.10), which contained water at least once during the study period and covered the majority of the wadeable area of the study site and totalling approximately 790 m^2. These were a deep headwater spring-fed pool, a shallow pool at the confluence between the previous habitat and an intermittent tributary, a deep pool with thick riparian shading of crack willow (*Salix fragilis*), a large wide deep pool used for duck shooting, and a shallow downstream habitat with in-stream flax (*Phormium tenex*) and *Carex secta*, which regularly dried up. These habitats were termed Spring Pool, Confluence, Willow Pool, Duck Pond, and Wetland respectively (Figure 1.11).

Figure 1.11. Habitats at Hororata Spring, including from upstream to downstream, the deep Spring Pool (A), the extensive Duck Pond with Mai Mai (B), the Willow Pool (C), and a shallow area by the Wetland after heavy grazing (D).

St Andrews Drain

General landscape

Figure 1.12. St Andrews Drain NZMS 260 J39 Timaru (236700E, 563120N) Topographical map (top) and orthograph (bottom). The main study site is within the circles. Grid is 1 km^2 in topographical map; north is at the top of the page in both views. The South Pacific Ocean is seen in the bottom right corner of both images.

St Andrews Drain is a small channalised spring-fed perennial watercourse, which is used for drainage, stockwater and irrigation (Figure 1.12). Eldon (1993, p. 8) stated that 'mudfish were once very abundant in this waterway. In October 1982, it was possible to obtain 50 fish in a matter of minutes, using a pole net'. The site was located within one kilometre of the South Pacific Ocean, however the waterway does not reach the sea, terminating instead in a lagoon area created by a high gravel bar. This site would once have been a shallow wetland area, a paleo-channel is still evident and wetland plant species (e.g. *Juncus* spp.) still persist, providing a glimpse of what the area must once have looked like (Figure 1.13).

The site was predominantly grazed by sheep; however beef cattle were bought onto the property in 2002. Deterioration of easily accessed areas of the waterway was observed after cattle introduction. Mechanical clearance of in-stream vegetation occurs, though infrequently, at which time Department of Conservation staff are often present to salvage fish. The channelised section has short steep banks bounded by material that has been removed from the stream bed. These mounds likely protect the waterway from direct surface runoff from surrounding paddocks. *G. breviceps* were sometimes caught at this site, but their low abundance (< 10) and size structure indicated that a viable population was not present.

Habitats sampled within St Andrews Drain

Four habitats totalling 460 m^2 were sampled; these included the stream's headwaters, where ground water springs, fed from surrounding hills, emerged; a shallow, wide pool above a culvert, which was often the only area of open water; a deep macrophyte dominated channalised section below this culvert; and a section of stream at the bottom of the study site, where the channel was wide and reminiscent of the historic shallow wetland, with multiple channels forming around *Juncus* spp. clumps (Figure 1.14). These habitats were termed Spring, Pool, Ditch and Lower, respectively.

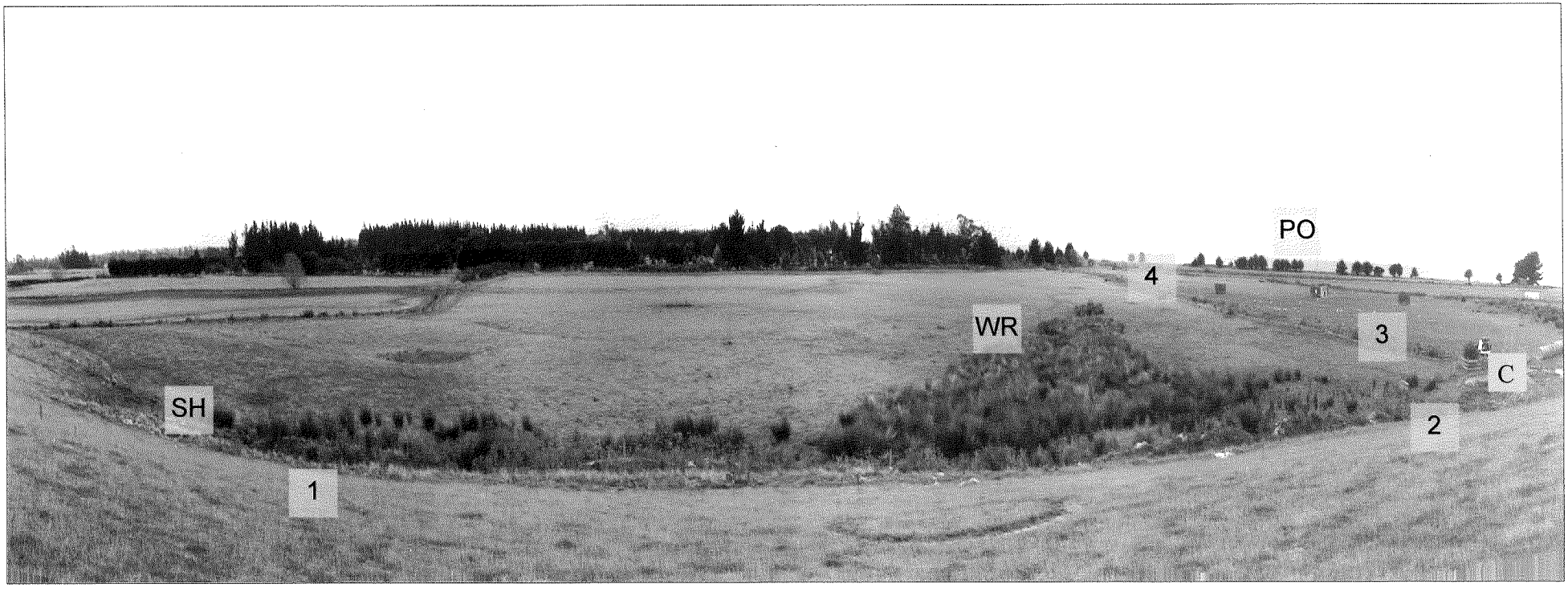

Figure 1.13. Approximate locations of habitats intensively studied at St Andrews Drain where 1 = Spring, 2 = Pool, 3 = Ditch, and 4 = Lower. SH = Seepage spring from hill on which this photo was taken, WR = remnant wetland vegetation that is likely to indicate original path of drainage, C = location of culvert, PO = South Pacific Ocean.

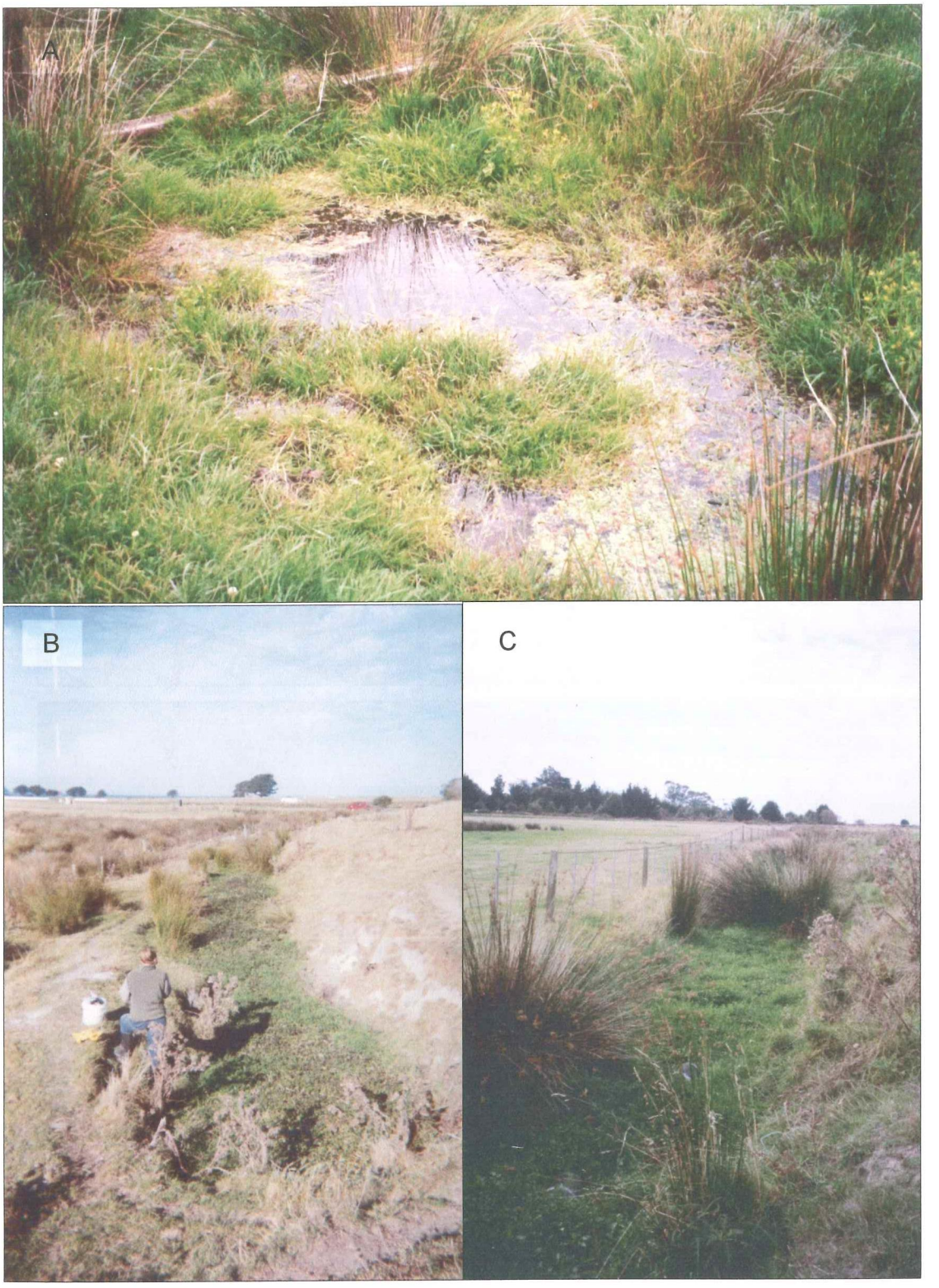

Figure 1.14. Habitats at St Andrews Drain, including the Lower area (A), the Spring (B) where groundwater emerges and is channalised along the base of a low hill, and the Ditch (C). These latter habitats were usually overgrown with macrophytes during the study.

Dog Kennel Stream

General landscape

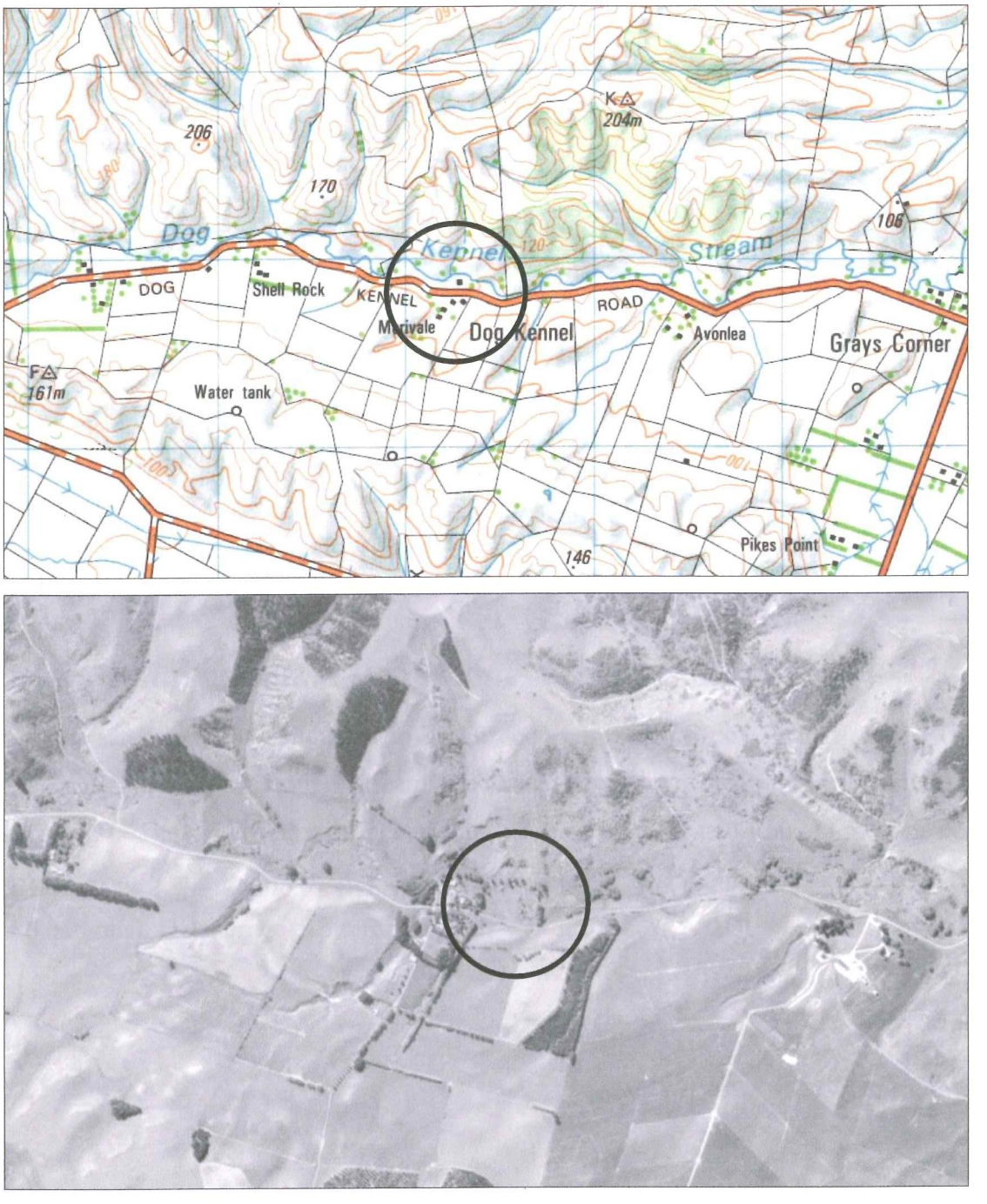

Figure 1.15. Dog Kennel Stream NZMS 260 J40 Waimate (23544E, 55928N) Topographical map (top) and orthograph (bottom). The main study site is within the circles. Grid is 1 km^2 in topographical map; north is at the top of the page in both views.

Dog Kennel Stream consists of a series of steep-sided pools, which are intermittently connected by flow, situated in a hilly catchment (Figure 1.15). Approximately 2 km of waterway has been placed under legal conservation covenant, protecting the area from further modification, and is managed by the Department of Conservation (Gray 2000; Harraway 2000). Riparian plantings of *P. tenex*, cabbage tree (*Cordyline australis*) and *Coprosma* spp. were conducted in 1999. This habitat receives little direct agricultural pressure as it has been fenced, and only the occasional sheep grazes near the pools.

Eldon (1979a) described *N. burrowsius* occurring in seepage streams that 'were of low volume (estimated at < 5 litre·s^{-1} normal flow), but contained water all year round, they were characterised by alternating steep-sided pools of 1.5 m or more in depth covered with floating vegetation, and weed choked shallows. A typical example was Dog Kennel Stream, south of Waimate. In the upper reaches deep pools covered with floating vegetation contained permanent water, though flow ceased at times. The lower reaches had been channelled, and were dry during most of the study' (Eldon 1979a, p. 113).

Habitats sampled within Dog Kennel Stream

Long-term sampling was conducted in seven deep pools in the covenanted section (Figure 1.16); termed Pools 1 – 7, from upstream to downstream, all of which provided similar habitat (Figure 1.17). The surface area of these pools at full capacity ranged from 34 m^2 for Pool 7 to 93 m^2 for Pool 5 and the mean size was 51 m^2. The greatest depth measured was 2.1 m in Pool 6, although the average depth for all pools was 1.1 m.

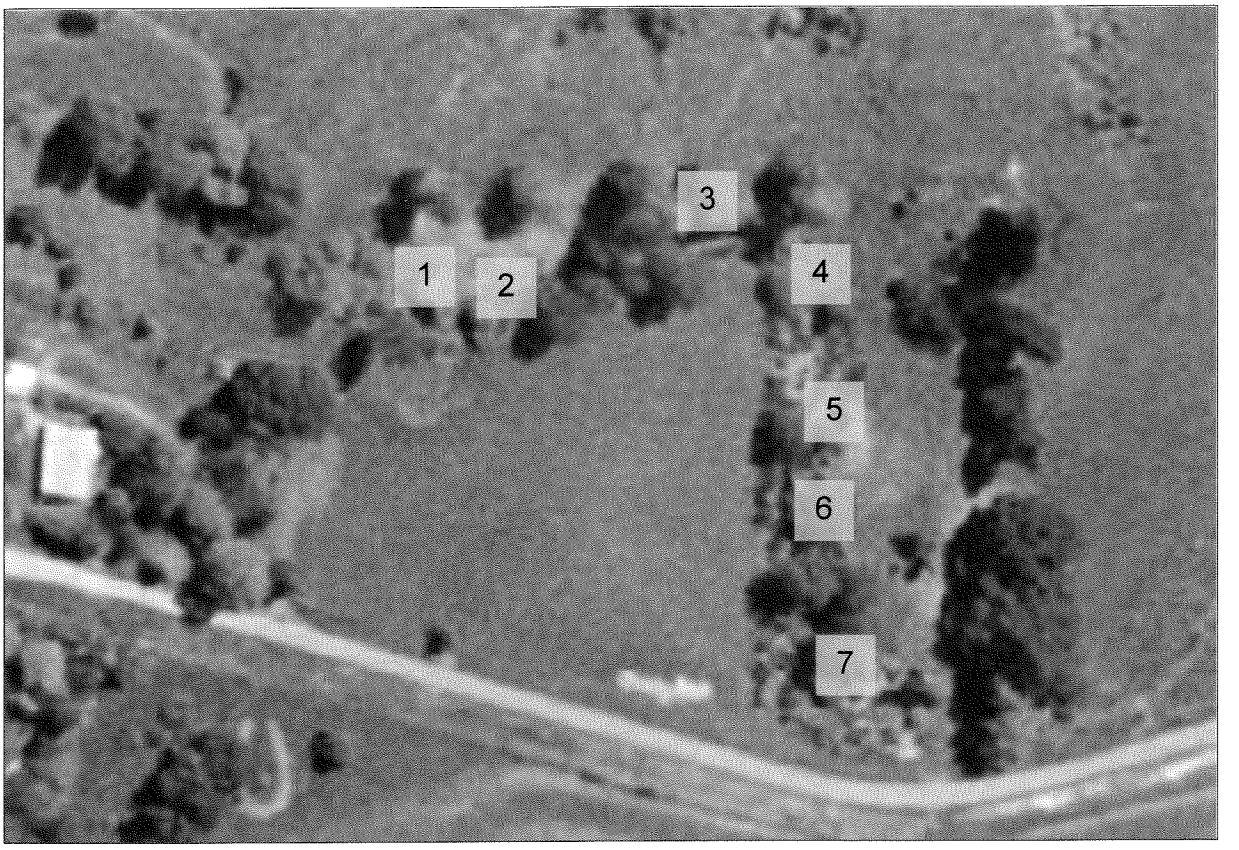

Figure 1.16. Approximate location of habitats frequently sampled in the covenanted section of Dog Kennel Stream. Pool habitats were numbered 1 – 7 from upstream to downstream.

Figure 1.17. Pools at Dog Kennel Stream during 1999 (A) and 2002 (B – D). Most of the flax and cabbage trees were planted.

SECTION 1

Methods of capture, monitoring, and habitat assessment

Sampling at Hororata Spring. At top, Gee minnow trapping during summer 1999 in the Duck Pond. At bottom, electrofishing in the downstream Wetland habitat during autumn 2002.

Chapter 2.

Active and passive methods used for the capture of *Neochanna burrowsius*.

INTRODUCTION

To conserve a threatened species, it is important to evaluate its distribution and abundance using reliable measures. Unfortunately, as a species becomes increasingly rare, it becomes more difficult to detect and sample adequately (Green & Young 1993). To further compound this dilemma, the genus *Neochanna* is notoriously difficult to catch, although the focus of this study, Canterbury mudfish (*N. burrowsius*) is considered 'by far the easiest mudfish to capture' (Eldon 1992, p. 17). The nocturnal, cryptic habits of *Neochanna*, coupled with the soft mud and thick vegetation of their wetland habitats, which acts to conceal fish and create challenging sampling conditions, makes capture difficult (Eldon 1992). Further, wetlands are often composed of both lotic and lentic environments that provide a vast range of habitats, differing in depth, vegetation cover and hydrologic dynamics (Partridge 1982). Thus, selecting a method of fish capture that is equally effective in such a range of habitats is problematic due to differences in the performance of sampling techniques.

A wide variety of methods for catching fish have been developed, which is a reflection of the diversity of fish behaviours and the variability in the effectiveness of different methods in dissimilar habitats. Fish capture techniques can be broadly categorised as employing either passive or active methods. The applicability of a particular method requires several considerations, including efficiency, effectiveness, operator safety and degree of habitat disturbance. Additionally, there are logistical limits to the extent of habitat that can be surveyed. Passive methods of fish capture, such as set-netting and trapping, are often constrained by the amount of sampling gear available (Ryan & Kerekes 1989), whereas, many active methods, such as electrofishing and seining, are usually limited to wadeable waters (McDowall 1990).

Passive sampling methods

Passive sampling methods rely on fish moving into a trap or becoming entangled in a fine mesh. This chapter focuses on the use of Gee minnow traps (GMT; Cuba Speciality Manufacturing Company, Fillmore, New York, USA), which have been widely utilised in New Zealand as a method of capturing *Neochanna*, e.g. Eldon (1992), and Hicks & Barrier (1996). As such, GMT are considered to be the most reliable technique for capturing all *Neochanna* species (Ling et al. unpublished). However, few tests of the effectiveness of GMT have been performed.

Capture rates by passive fishing gear, such as GMT, are influenced by the amount fish are foraging, and characteristics of the weather and habitat (Hamley & Howley 1985; Ryan & Kerekes 1989; Barrier et al. 1996). Fish foraging behaviour is influenced by the availability of food resources and the presence of competitors or predators (Mittelbach & Chesson 1987; Sih 1992). Furthermore, factors, such as mesh size, can change the effectiveness and size-selectivity of passive gear (Hayes 1989). Although the effect of mesh size has been investigated for other types of passive gear such as set-nets (e.g. Studholme 2002), few studies have compared the influence of mesh size on GMT capture success. However, Dean (1995) suggested that differences in the size and number of black mudfish (*N. diversus*) captured between two sampling occasions was due to the subsequent additional use of fine mesh traps. I investigated the influence of trap mesh size, habitat, and the presence of other fish on the number of *N. burrowsius*.

Active sampling methods

Active sampling methods include physical techniques, such as sweep netting, herding fish, and the application of an electrical current to elicit a forced swimming response (galvanotaxis; McDowall 1990). Electrofishing machines (EFM) are widely used in fish capture. A large variety of EFM sampling protocols have been employed, depending on fish behaviour and habitat type (McDowall 1990; Heimbuch et al. 1997), and have even included 'throwing electrodes' to capture highly mobile fish (Persat 1991).

Generally, the capture of *Neochanna* by EFM relies on fish being affected by the electrical current in a way that enables movement to be visually detected. In turn, the effectiveness of the electrical current depends on water conductivity and substratum characteristics. Movement by fish depends on factors such as cover, particularly if fish bury themselves

within mud, which has electrical insulating properties (Eldon 1992). Visual detection depends on water clarity and the density of macrophytes. Once movement is detected, capture relies on the reflexes and experience of operators using dip-nets. Further, the probability of fish capture by EFM often increases with fish size (Anderson 1995). This is due to larger fish being affected to a greater extent by the electric field, and consequently an increased ability of operators to see and capture them.

Combined active and passive sampling methods

Fish surveys in lentic habitats using only one sampling method have been found to underestimate species richness and abundance, and to introduce size-selective bias (Anderson 1995; Jackson & Harvey 1997). Thus, utilisation of a variety of passive sampling methods is commonly recommended for surveys of lentic habitats. Such a procedure also enables the comparison and assessment of sampling gear efficiency (Hayes 1989; Jackson & Harvey 1997; Studholme 2002). GMT have been used in association with EFM in fish surveys in New Zealand (Allibone & Chadderton 1992). However, few studies in New Zealand or overseas have included detailed and direct comparisons of the effectiveness of active and passive methods used in combination. Notwithstanding this, Hayes (1989) compared six fishing methods and concluded that active fishing gear (beach seine) sampled small littoral fish more representatively than many passive types of gear.

In my study, initial sampling indicated that GMT caught substantially fewer *N. burrowsius* in some habitats than were known to occur from direct visual observations (Chapter 7). Electrofishing was then trialled as it had previously been used to survey *N. burrowsius* and brown mudfish (*N. apoda*), despite possible limitations in some habitats (Cadwallader 1973; Eldon 1968, 1978, 1979a, 1992; Eldon et al. 1978). Clearly, there was a need to conduct comparisons of these two methods in combination, in a variety of habitats, to determine their value for *N. burrowsius* capture. The purpose of my investigation was thus to determine an effective method of *N. burrowsius* capture and to evaluate the relative effectiveness of active and passive methods in four remnant wetlands. These four sites differed in their hydrological, morphological, biotic, and vegetation characteristics. These factors are likely to affect the efficiency and effectiveness of fish capture methods.

METHODS

I sampled four sites identified by Eldon (1993) as important natural habitats containing *N. burrowsius*, and that encompassed a wide variety of conditions. Te Roto Repo o Tawera comprised of a small perennially flowing stream system running through remnant wetland vegetation. Hororata Spring encompassed a large ephemeral spring system consisting of deep pools, predominantly. St Andrews Drain was a small spring-fed stream heavily overgrown with macrophytes. Whereas, Dog Kennel Stream consisted of a series of intermittently connected deep pools (see Chapter 1 for more detailed descriptions of sites). Within each of the four sites, four to seven distinctive habitats were identified. The size of habitats sampled was estimated by taking between three and five width and depth measurements within each habitat. Water depth was measured using a metre ruler in shallow waterways and a weighted tape measure in deeper pools. Wetted surface areas were calculated assuming a rectangle for run type habitats and ellipses in pool habitats. All fish captured were anesthetised with approximately 0.5 x 10^{-5} g/l of 2-phenoxyethanol in stream water before being weighed and measured. Fish were then placed in a recovery bucket containing aerated stream water and preventative treatment consisting of ~ 5 ml Aqua Plus® and ~ 1 ml Spot Off®. Aqua Plus® is a commercial water conditioner containing polymers, pure herbal extracts, and essential oils. This is purported to have a sedative effect against stress related to transportation and handling, as well as, protecting fish by replacing their natural mucous coating. A similar water conditioner (NovAqua®) has been shown to significantly increase survival during transportation in delta smelt (*Hypomesus transpacificus*; Swanson et al. 1996). Spot Off® was a previously available, wide spectrum bactericide, containing 0.01 % acriflavine, 0.1 % malachite green and 0.05 % formaldehyde. Fish were then re-released into the same habitat from which they were caught.

GMT

Passive sampling was conducted using GMT constructed from 6 x 6 mm ($^1/_4$") wire mesh with approximately 30 mm diameter entrances. After preliminary observations of capture effectiveness, GMT were covered with 2 x 2 mm black plastic mesh. This also reduced injury, as *Neochanna* can become wedged in the larger mesh (Dean 1995; personal observation). GMT were baited with Marmite® (Sanitarium Health Food Company, New Zealand), a yeast extract based food spread, following the method of Allibone & Chadderton

(1992). GMT were secured near the water surface, especially in lentic conditions, and left overnight (~ 16 h).

EFM

Electrofishing was conducted using a Kainga EFM 300 (NIWA Instrument Systems, New Zealand) backpack electrofishing machine fitted with a 450 mm diameter heart-shaped anode set to produce pulsed DC current (pulse width ~ 3 ms, 60 pulses/s). High conductivity occurred when sampling sites where disturbed, which often resulted in 100 V DC producing an output current of 1 amp. This current is higher than usually used; however, it effectively induced forced swimming in *N. burrowsius*. Further, a strong electric field may be more effective for capturing small fish (NIWA unpublished). Spinal damage or other deleterious effects were never observed in fish. All electrofishing was conducted by a single operator in the water, with a second on the bank to reduce habitat damage. EFM effort was measured as the number of minutes the electrical current was passed through the water, as recorded by the EFM.

Development of an appropriate EFM technique was an important step in sampling *N. burrowsius* populations. This was because most *N. burrowsius* habitats were essentially lentic, thus, techniques such as fishing downstream to a block-net held by a co-worker, were impractical. Several techniques appeared to improve capture rates of *N. burrowsius* by EFM. Most importantly, the operator moved very slowly so that any fish movement was detected and sediment disturbance was reduced (Eldon 1992). In areas overgrown with submerged and emergent macrophytes, sweeping the electrode from side to side through habitat was impossible. Instead, the electrode was placed on the water surface, a current applied and the electrode slowly pushed down against the macrophytes. Affected fish were captured, as they came to the surface, using dip nets, which were also regularly swept around areas under the influence of both the anode and cathode. *N. burrowsius* were more readily captured after a second short application of electrical current, as also observed by Eldon (1992), when sampling *N. apoda*.

GMT mesh size comparison

The effectiveness of GMT with differing mesh size was investigated at St Andrews Drain and Dog Kennel Stream in autumn 1999. The number of *N. burrowsius* captured using GMT with

6 x 6 mm mesh was compared against that caught using modified GMT with 2 x 2 mm mesh. Eighteen GMT with small mesh and twenty with large mesh were placed randomly in habitats for one trapping night at St Andrews Drain and two nights at Dog Kennel Stream.

Size-selectivity of GMT and EFM

A comparison of the size-selective capture of *N. burrowsius* by GMT and EFM was conducted at Te Roto Repo o Tawera in April 1999, using the Top, Middle and Bottom reaches of the main stream (see Chapter 1 for further descriptions). One-pass EFM sampling was conducted on 7 April 1999 in each reach. After measurement, fish were returned to the reach from which they were caught. Electrofishing has been shown to change fish behaviour for 24 hours (Cross & Stott 1975; Mesa & Shreck 1989). Thus, fish were left for 48 hours to recover before subsequent sampling using GMT on 9 April 1999, with six traps placed in each of the three previously electrofished reaches. This comparison also evaluates capture rates of EFM and GMT.

Combined GMT and EFM removal methods

A combined removal method involving the retention of fish captured overnight by six GMT followed by three-pass EFM sampling the next day was tested in autumn 2001 at Te Roto Repo o Tawera. Stop-nets spanning the width of the stream were placed at either end of a 50 m section before trapping and left until after electrofishing was completed. This test allowed the effectiveness of the passive GMT to be evaluated by determining the number of fish subsequently captured using an active method.

The initial trial using the combined removal method at Te Roto Repo o Tawera was effective at capturing large numbers of *N. burrowsius*. Thus, in 2002 a removal method involving overnight GMT followed by a single pass of EFM was conducted in 21 habitats amongst the four study sites. Time constraints imposed by the intensive sampling method restricted the number of passes that could be conducted at each habitat within a site within 24 hours. The use of single pass EFM has been investigated by Jowett & Richardson (1996) in streams, where they captured an estimated 51 % of the fish population present. In my study, all sites were sampled (within an eight day period) in this manner twice, one month apart, during autumn (April and May). Stop-nets were placed at the top and bottom of lotic sites. To

compare relative density of fish captured by each method, CPUE was multiplied by the sampling intensity at each habitat (Table 2.1).

During April 2002, the pools at Dog Kennel Stream were covered with the free-floating macrophytes duck weed (*Lemna minor*), Pacific azolla (*Azolla filiculoides*), and water meal (*Wolffia australiana*). During electrofishing these floating macrophytes were pushed aside to improve visibility. However, heavy rain caused flooding in South Canterbury two weeks before the May sampling and removed most of this floating cover (M. & H. Redshaw, previously Lyall Rd, St Andrews, personal communication). Due to the change in visibility and available cover for fish, it was hypothesised that the presence or absence of floating aquatic vegetation would change the relationship between EFM and GMT capture rates.

Table 2.1. Summary of sampling measures calculated and compared in this chapter using Gee minnow traps (GMT) and an electrofishing machine (EFM).

	GMT	EFM
Effort	Number of traps placed in a habitat overnight (unit = 1 trap)	Number of minutes of electrical current passed through a habitat (unit = 1 minute)
Intensity	Effort used in each habitat (traps/m^2)	Effort used in each habitat (minutes/m^2)
Catch per unit effort (CPUE)	*N. burrowsius* caught per number of traps placed in habitat (*N. burrowsius*/traps)	*N. burrowsius* caught per time spent in habitat (*N. burrowsius*/minutes)
Capture density	CPUE x Intensity (*N. burrowsius*/m^2)	CPUE x Intensity (*N. burrowsius*/m^2)

Statistical analysis

Statistical analysis on data comparing capture of small and large mesh GMT was conducted using a Kruskal-Wallis test as data could not be normalised due to some extreme values and many empty traps. Such a situation is often found with CPUE data obtained by passive methods (Hamley & Howley 1985).

The size-selectivity of each method was investigated using size – frequency histograms with size class widths of 5 mm, following the convention described in Nielsen & Johnson (1983). The size – frequency histograms of *N. burrowsius* captured by each method (GMT and EMF) were then compared using a Chi-squared test of independence.

As it was likely that the number of fish captured by EFM would be dependant on the number of fish already removed by GMT, densities of fish captured by each method were compared by regression analysis. Comparison of the relationship between passive and active capture in the presence or absence of free-floating vegetation was conducted using an homogeneity of slopes test and ANCOVA in Prism 3.0 (GraphPad Software 1999).

The influence of the presence of other fish species on the capture rate of *N. burrowsius* in passive GMT was tested by factorial ANOVA. The proportion of total *N. burrowsius* caught by GMT in each habitat containing upland bully (*Gobiomorphus breviceps*) and/or shortfin eel (*Anguilla australis*), or neither species, amongst all habitats was arcsine transformed.

RESULTS

GMT mesh size comparison

Mesh size had a large influence on the effectiveness of GMT in capturing *N. burrowsius*. GMT with small (2 x 2 mm) mesh were more likely to capture *N. burrowsius* than 6 x 6 mm mesh GMT, with *N. burrowsius* captured in 54 % (n = 29) of small mesh GMT, but in only 15 % (n = 9) of large mesh GMT. Furthermore, small mesh GMT caught a greater number of *N. burrowsius* (Kruskal-Wallis ANOVA by ranks test: $H_{(1, N = 101)} = 16.4$, $p < 0.001$, Figure 2.1). Due to this result only small mesh GMT were subsequently used.

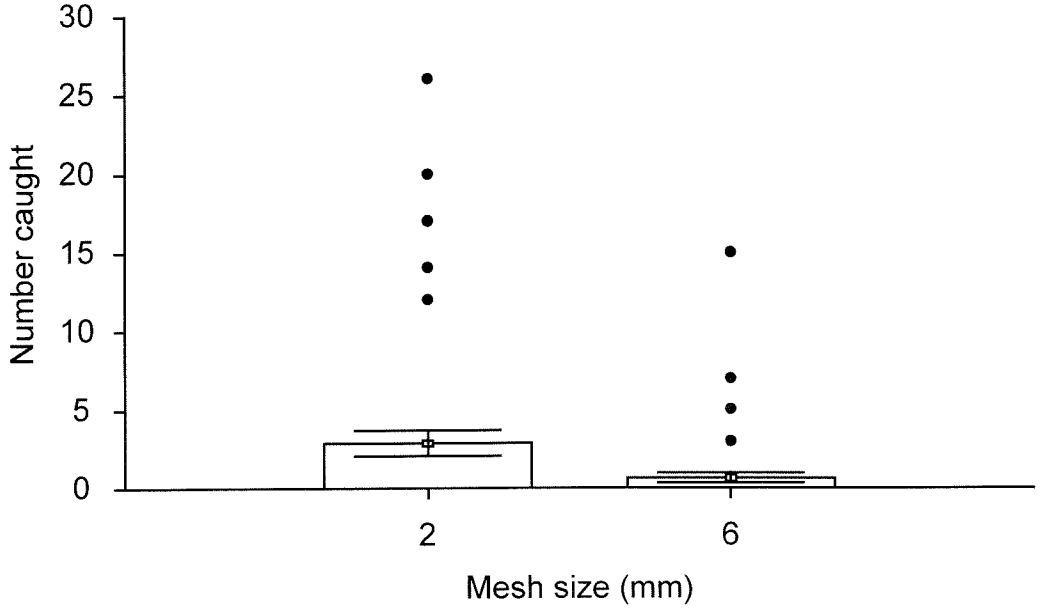

Figure 2.1. Numbers of *N. burrowsius* caught overnight in individual traps with 2 x 2 mm and 6 x 6 mm mesh. GMT of both type were placed randomly in St Andrews Drain and Dog Kennel Stream. Bars indicate mean ± 1 SE, circular points indicate extreme values. Data from traps that did not capture fish were not included.

Size-selectivity

The comparison conducted at Te Roto Repo o Tawera between GMT and EFM methods indicated that there were no significant differences in the size of fish captured by the two methods (Figure 2.2; χ^2 = 19.7, df = 35, p = 0.9). EFM also captured nearly four times as many *N. burrowsius*.

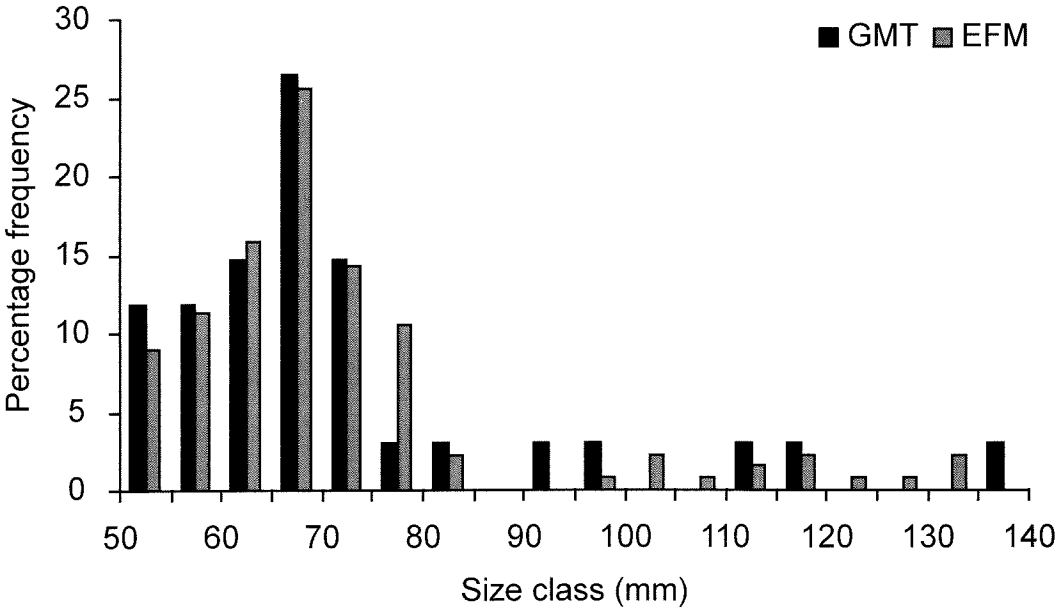

Figure 2.2. Percentage frequency of *N. burrowsius* caught in each size class using Gee minnow traps (GMT) and electrofishing (EFM) methods. GMT and EFM captured 34 and 133 *N. burrowsius*, respectively.

Combined GMT and EFM removal method

Evaluation of a removal protocol using passive GMT, followed by active EFM methods conducted at Te Roto Repo o Tawera, indicated that the combined capture method was more effective than Gee minnow trapping alone. GMT captured only 45 % of the total *N. burrowsius* caught within the reach (Figure 2.3). Whereas, trapping followed by one pass of EFM captured 77 % of the total *N. burrowsius* caught. Thus, the use of GMT followed by EFM substantially increased the number of *N. burrowsius* it was possible to catch within a 24-hour period.

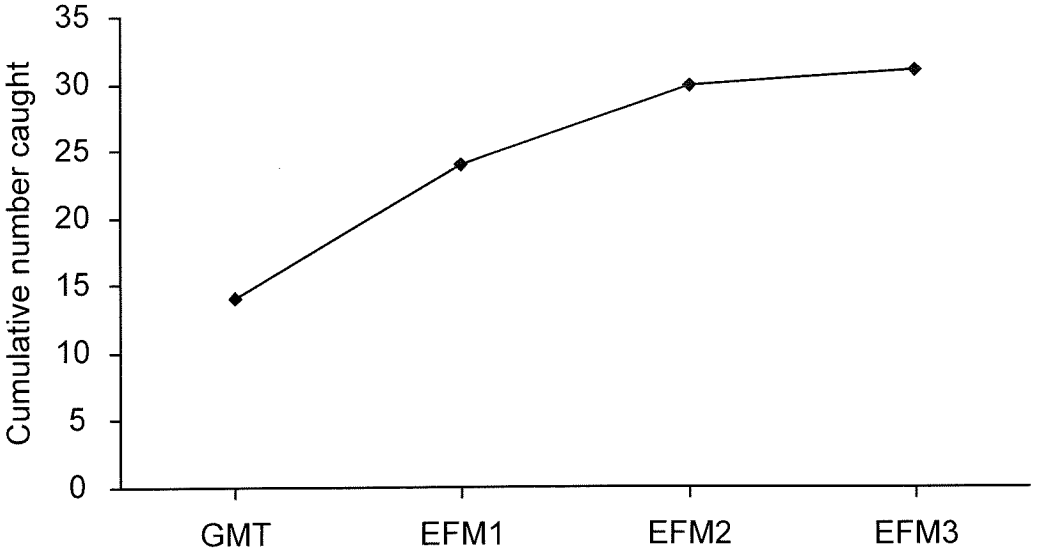

Figure 2.3. Cumulative number of *N. burrowsius* caught in a stop-netted 50 m reach at Te Roto Repo o Tawera using six Gee minnow traps (GMT) placed overnight, followed by 3-pass electrofishing (EFM 1 – 3) the next day.

Comparison of combined removal method among habitats

The relative effectiveness of GMT in differing habitats was assessed by comparison with the density of *N. burrowsius* subsequently caught using EFM. Here a shallow slope in the relationship between GMT and EFM capture density would indicate that GMT were most effective, capturing the majority of fish present, whereas a steep regression slope would indicate that the majority of fish were captured by subsequent EFM sampling.

Unlike all other sites, the densities caught by each method at Te Roto Repo o Tawera were significantly associated (Figure 2.4; $R^2 = 0.71$, $F_{(1, 6)} = 15$, $p < 0.01$), suggesting that both methods were effective throughout the site. In contrast, at St Andrews Drain the effectiveness of each method differed widely between habitats. In Hororata Spring very few of the total number of fish were caught by GMT (Figure 2.4).

Factors, such as site wadeability is unlikely to be associated with this pattern as the majority of fish were captured by EFM in Hororata Spring, despite many areas being relatively inaccessible. However, the presence of other fish species appeared to influence the proportion of *N. burrowsius* captured in GMT, as the lowest overall catch effectiveness was found at Hororata Spring, which contained both *A. australis* (n ~ 10) and *G. breviceps*. In particular,

the presence of *A. australis* may have suppressed activity in *N. burrowsius*, as a smaller proportion of *N. burrowsius* were captured by passive GMT than EMF, in their presence (Figure 2.5, Table 2.2).

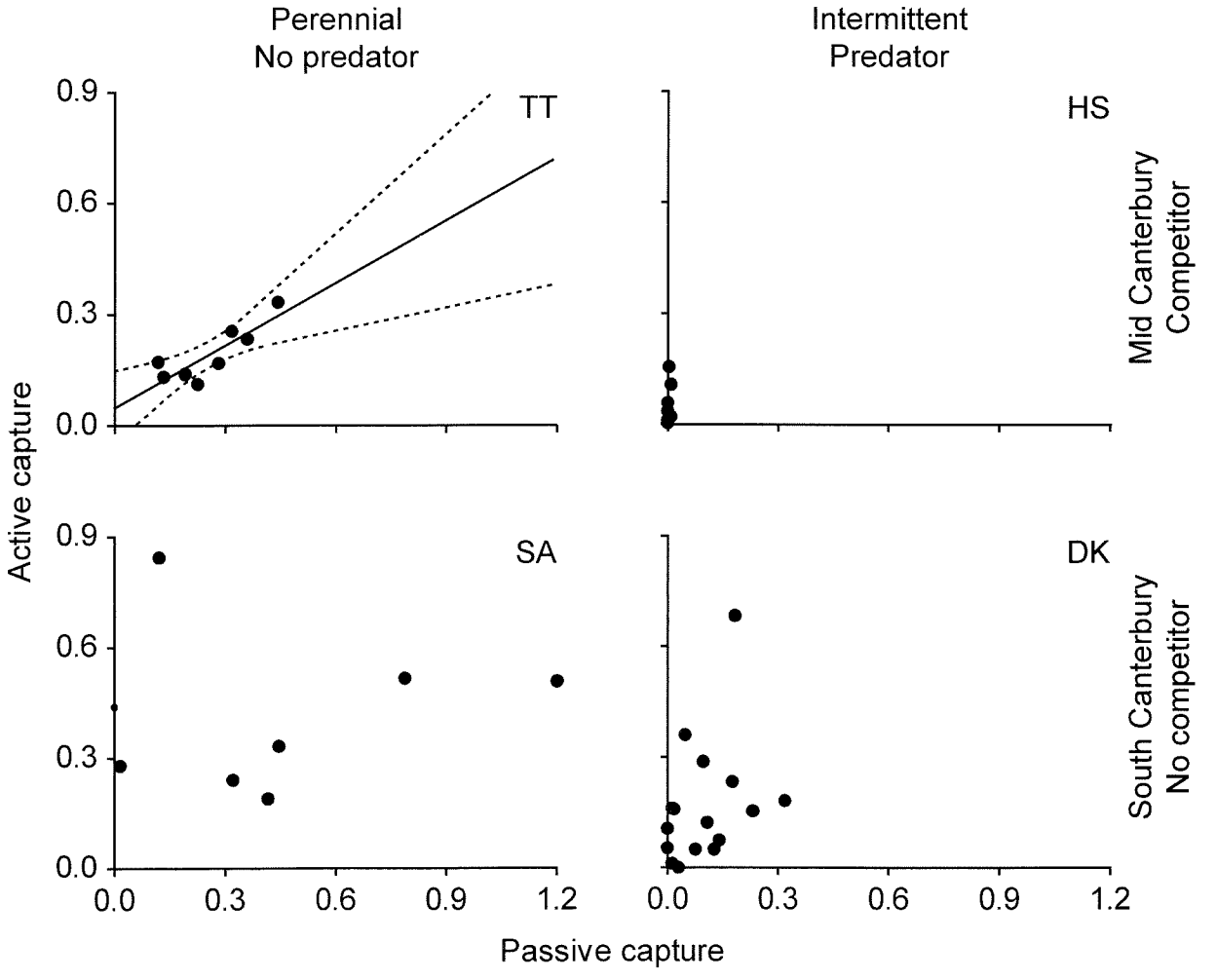

Figure 2.4. *N. burrowsius* caught per metre squared by initial GMT trapping regressed against *N. burrowsius* caught (per metre squared) by subsequent EFM sampling. Sites are TT = Te Roto Repo o Tawera; HS = Hororata Spring; SA = St Andrews Drain; DK = Dog Kennel Stream.

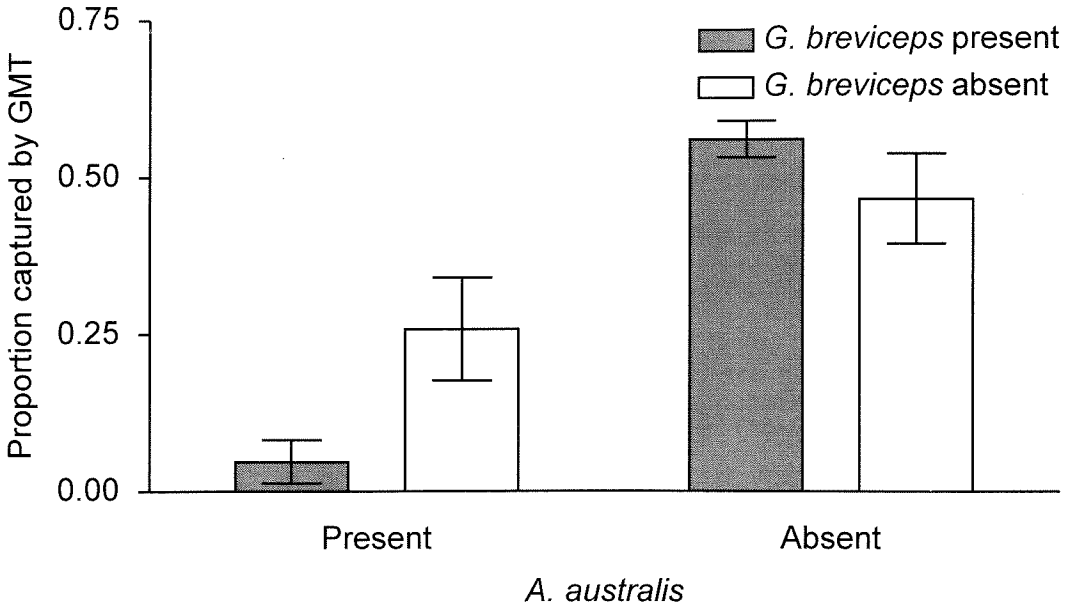

Figure 2.5. Mean (± 1 SE) proportion of the total *N. burrowsius* captured by passive Gee minnow traps (GMT) in the presence and absence of *G. breviceps* and *A. australis* within all habitats combined.

Table 2.2. Results of factorial ANOVA testing the influence of the presence and absence of *A. australis* and *G. breviceps* on the proportion of *N. burrowsius* captured by GMT in each habitat across all sites.

Source	df	MS	F-ratio	*p*-value
A. australis	1	3.05	10.8	0.002
G. breviceps	1	0.37	1.3	0.3
Interaction	1	0.03	0.1	0.8
Error	37	0.28		

Influence of floating aquatic plant cover

The presence of free-floating surface vegetation influenced the relative capture density of fish sampled by both passive and active methods at Dog Kennel Stream (Figure 2.6). Slopes describing the relationships in *N. burrowsius* density between GMT and EFM between April and May 2002 were significantly different (homogeneity of slopes test: $F_{(1, 12)} = 10.7$, $p = 0.007$). The steep slope in April, when sites were covered with free-floating vegetation,

indicates that a larger number of *N. burrowsius* were captured by EFM than GMT. Whereas the shallow slope during May, when floating vegetation had been removed by floods during the intervening period, indicates that GMT were more effective at capturing *N. burrowsius*, with few further fish being caught by EFM. This was despite reduced plant cover allowing good operator visibility in May. Further, there were no significant differences in the total numbers of *N. burrowsius* caught by both methods combined (ANOVA: $F_{(1, 14)} = 0.7$, $p = 0.4$) between April and May. Thus, although the two methods differed in effectiveness on the two occasions, similar numbers of *N. burrowsius* were captured in total.

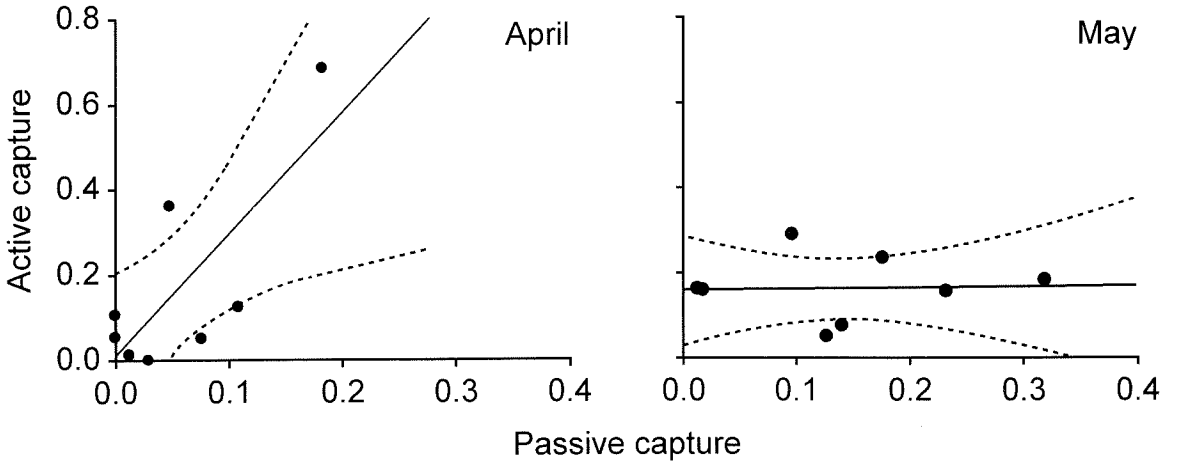

Figure 2.6. The relationship between initial GMT capture (number per metre squared) and subsequent EFM (number per metre squared) during April and May 2002, at Dog Kennel Stream. During April, pools were covered with free-floating vegetation, which was removed by flooding prior to the May sampling occasion.

DISCUSSION

The combined passive and active sampling protocol developed in this study greatly increased the number of *N. burrowsius* able to be captured in a 24 hour period. Further, although the use of small mesh GMT was generally an effective method of capturing *N. burrowsius*, in several habitats substantial numbers of additional fish were captured by EFM sampling. Several factors likely contributed to the observed differences in the effectiveness of GMT among sites. GMT were generally less effective in predominately lentic sites, such as Dog Kennel Stream and Hororata Spring, compared with the two slowly flowing sites. This pattern, may in part, be due to differences in the diffusion of bait (Marmite®) odour from traps, with water flow spreading odour over a greater area. However, the use of bait is considered by some workers to have little influence on the number of *Neochanna* captured (Hicks & Barrier 1996; Ling et al. unpublished). Further, Gee minnow trapping was less effective in habitats that were both shallow and overgrown with aquatic vegetation, or that contained *A. australis*. That GMT captured a lower proportion of *N. burrowsius* than EFM in overgrown habitat was unexpected. This finding is important because GMT and other passive methods are used extensively in lentic habitats dominated by macrophytes to capture *Neochanna*. Shallow and overgrown habitat may restrict the movement of *N. burrowsius,* thus reducing the numbers able to enter traps. However, this result may only relate to *N. burrowsius* and not other *Neochanna* species, which are more adapted to shallow wetland habitat (McDowall 1997a). Indeed, *N. apoda* were caught by Grainger (2000) in GMT dug into the ground, in near-terrestrial situations. However, subsequent EFM sampling, or retention and repeat trapping was not performed to test GMT effectiveness on *N. apoda*. Additionally, in shallow or otherwise restrictive habitats, the effectiveness of EFM may increase as opportunities for fish to escape are limited (Persat 1991).

Much of the criticism focused at EFM as a method for capturing *Neochanna* relates to operational difficulties, or as aptly put by Eldon (1968, p. 46) 'it is sometimes impossible to make a stealthy approach in the quaking mire of a swamp, and it is necessary for the quarry to be cornered or trapped for the machine to be effective'. The wetland remnants surveyed in the present study were all modified to some extent by agricultural activity, which undoubtedly facilitated the use of EFM. However, discounting EFM as a method of *Neochanna* capture may be premature as active methods do not rely on foraging behaviour. Further, the use of EFM forces *N. burrowsius* to move even in the presence of *A. australis*. This is of significance as GMT were less effective at sites that contained *A. australis*. *A. australis*

captured during this study were large and thus potentially piscivorous (minimum size 500 mm and maximum 850 mm). Behavioural changes, such as reduced activity, can be induced by the presence of predators, and have been documented widely in fish (e.g. Mittelbach & Chesson 1987; Lima & Dill 1990). Collins et al. (1983) also demonstrated that predatory fish reduce the susceptibility of crayfish to passive trapping gear, thereby influencing density estimates. Thus, it is likely that the presence of large piscivorous *A. australis* reduced the activity of *N. burrowsius*, and thus, lowered the probability of capture by GMT. This finding emphasises the importance of ascertaining whether *A. australis* is present in a site when assessing the presence and/or abundance of *N. burrowsius* using GMT alone.

It was hypothesised that a dense cover of free-floating vegetation would reduce operator visibility, thus reducing the number of *N. burrowsius* caught by active methods. Contrary to this expectation, EFM was more effective when a surface layer of free-floating vegetation was present. There are three possible explanations for this increased effectiveness relating to the use of cover, foraging activity, and behavioural changes due to previous experience of electrical fields. It is possible that dense overhead cover, such as that provided by free-floating vegetation reduces the need for *N. burrowsius* to seek benthic refuge to avoid avian predators. If so, the effectiveness of EFM is likely to be greater if fish are close to the water surface, or not insulated by mud substratum (Eldon 1978, 1992). Alternatively, high capture rates obtained by the passive method in the absence of free-floating macrophytes may indicate greater mobility and foraging activity after the flood event in Dog Kennel Stream. Food resources are often reduced during flooding (Sagar 1986). This may have required *N. burrowsius* to forage more, and thus may have been more likely to enter GMT. Finally, the low EFM catch rate in May could have been due to changes in behaviour induced by previous sampling, a phenomenon similar to trap shyness. Although this mechanism cannot be ruled out, studies on cutthroat trout (*Oncorhynchus clarki*), roach (*Rutilus rutilus*), gudgeon (*Gobio gobio*) and rudd (*Scardinius erythrophthalmus*) found that behavioural changes induced by EFM sampling lasted for less than 24 hours (Cross & Stott 1975; Mesa & Shreck 1989). Thus, it is most likely that the observed differences in GMT and EFM effectiveness between April and May were related to subtle changes in *N. burrowsius* foraging behaviour and cover use.

In the present study habitat-dependent differences in the effectiveness of active and passive methods were found. As the effectiveness of each method was dependent on different factors, the total combined abundance of both methods may represent a relatively unbiased estimate.

The use of active methods is also likely to reduce the large variability in CPUE typical of passive methods (Bagenal 1972; Hamley & Howley 1985). Importantly, no difference in size selectivity was found when the two methods were compared. Thus, I suggest that the abundance values obtained by each method may be added without weighting, as is employed when a variety of set-nets are used (Jackson & Harvey 1997).

The use of both active and passive methods not only allowed a general comparison of their effectiveness to be made, but also indicated differences in *N. burrowsius* behaviour in different situations. Such differences could enable hypotheses to be generated regarding the influence of habitat on *N. burrowsius* populations, via restrictions on movement and the presence of cover. Importantly, activity, potentially related to foraging by *N. burrowsius*, appears to be limited in the presence of *A. australis*, which may have consequences for growth and thus, fecundity of *N. burrowsius*. In summary, use of passive sampling methods that rely on aspects of fish behaviour, in combination with active methods that do not, can lead to robust abundance estimates for *N. burrowsius*, as well as a deeper understanding of factors influencing populations of wetland fish.

Chapter 3.

Methods of characterising microhabitat use of *Neochanna burrowsius*.

INTRODUCTION

Characterising optimal or preferred habitat, especially for threatened species, is an important component of ecological study (McCoy & Bell 1991; Bond & Lake 2003). Unfortunately, this can be hindered as threatened species are often poorly studied (LeCren 1990; Maitland 1995; Johnston 1999). In addition, organisms may aggregate in response to habitat heterogeneity at scales, and in ways, which investigators fail to perceive (Downing 1991). Thus, it is often difficult to decide *a priori* which habitat characteristics to sample and what level of detail needs to be recorded. Too coarse a scale of characterisation may mask important patterns of association, yet too fine a scale may introduce a high level of variability into analyses, as well as being time consuming. Hence, scale is a crucial concept in ecology, which can influence the results obtained, and the questions able to be answered (Weins 1989). These issues are widely recognised and it has been emphasised that scale needs to be defined clearly. Kotliar & Wiens (1990) detailed the importance of determining the 'grain' and 'extent' at which an organism responds to environmental patterns. Further, Dungan et al. (2002) noted that the concepts of scale involved in any study can be placed into three categories relating to the scale of (i) the phenomenon under study, (ii) the method of sampling, and (iii) the analyses conducted; with each category requiring treatment as a separate subject. Thus, in addition to environmental scale and sampling design, the characteristics of variables used in statistical analysis have an aspect of scale that needs to be recognised.

A further aspect involved in determining habitat – species associations is the choice of habitat type sampled. Studies into habitat preferences of New Zealand freshwater fish have predominantly involved measurements of water depth, velocity, and substratum composition in streams (e.g. Jowett & Richardson 1995; Allibone & Townsend 1997a; Broad et al. 2001; Baker et al. 2003). However, in lentic and wetland habitats these variables are less suitable as

water velocity, if any, and substratum are often similar throughout a site, limiting the usefulness of these measures as a predictor of small scale fish occurrence. In contrast, studies into the habitat associations of New Zealand wetland fish species, such as *Neochanna*, have tended to focus on vegetation and land use characteristics. Hicks & Barrier (1996) used wetland vegetation to generate a 'disturbance scale rating' reflecting the extent of human disturbance and presence of invasive plants in wetland remnants containing black mudfish (*N. diversus*). In comparison, Grainger (2000) surveyed the microhabitat within 1 m of individual Gee minnow traps, recording the abundance of all wetland plant species present, to investigate the effect of fire on the distribution of brown mudfish (*Neochanna apoda*).

Aquatic macrophytes have been used as limnological bio-indicators (Lehmann & Lachavanne 1999). This is because macrophytes effectively integrate environmental conditions over a long time frame. Thus, they can be used in a similar way to macroinvertebrates, as indicators of stream 'health' (e.g. Stark 1985). Plant species can be categorised according to their sensitivity to water quality and stream morphology (Seddon 1972; Dawson & Szoszkiewicz 1999), as well as, their ability to tolerate disturbance and resource limitation (Riis & Biggs 2001). Macrophytes can also change many small scale stream characteristics, such as flow, dissolved oxygen, and nutrient dynamics, often in a species-specific way (Seddon 1972; Howard-Williams et al. 1982; Cooper & Cooke 1984; Wilcock et al. 1995; Sand-Jensen & Pedersen 1999). Thus, detailing the composition of aquatic plant communities is likely to be an informative method of characterising the microhabitat of Canterbury mudfish (*Neochanna burrowsius*).

In-stream vegetation is largely restricted to slower flowing lowland streams that have some soft sediment (Riis & Biggs 2001). In such streams, macrophytes can provide most of the available habitat structure, and are a ubiquitous feature of the habitats which *N. burrowsius* occupies. Macrophytes are especially important for *N. burrowsius* as females scatter their eggs amongst in-stream vegetation at the water surface (Eldon 1979a, c). In this chapter I examine whether the occurrence of *N. burrowsius* at a scale of 1 – 10 m relates to course-scale descriptions of microhabitat, such as the proportion of macrophyte cover, or to fine-scale descriptions of microhabitat based on the occurrence of specific plant species. My aim was to determine which of these two approaches is likely to be most useful in identifying potential, and monitoring present, *N. burrowsius* habitat. The first method of habitat characterisation involved the assessment of the proportional occurrence of habitat

components, whereas the second approach involved the identification of each plant species present and the recording of detailed vegetation characteristics.

METHODS

During the austral autumn of 1999 a microhabitat survey was conducted at four sites containing *N. burrowsius* (See Chapter 1 for site descriptions). Vegetation characteristics varied both within, and between these sites, which were collectively representative of the habitat available to *N. burrowsius*. At each site, microhabitat within one metre of eighteen randomly selected sampling points was assessed. At each sampling point, a baited Gee minnow trap (2 x 2 mm mesh) was placed near the water surface and left overnight. In this chapter only data relating to the presence or absence of *N. burrowsius* in traps were used. See Chapter 2 for further details on fish handling procedure.

Assessment of microhabitat was conducted using a 0.5 x 0.5 m quadrate divided into 25 subunits. The quadrate was placed randomly within one metre of each Gee minnow trap in microhabitat similar to that in which the trap had been placed. All plant species present and their dominance in each quadrate subunit were recorded. Further estimates were taken of the percentage cover of riparian species greater than one metre high directly surrounding the sampling point, as well as, the percentage cover of submerged tree branches within each quadrate.

Taxonomic resolution of macrophyte species to genus was feasible in the field and was used in statistical analysis. Thus, many buttercups (*Ranunculus* spp.), e.g. celery buttercup (*R. sceleratus*), water buttercup (*R. trichophyllus*), and creeping buttercup (*R. repens*); were grouped together, although *R. trichophyllus* occurred most commonly. No distinction was made between the two watercress species *Rorippa microphylla* and *R. nasturtium-aquaticum*. Several sweetgrass' (*Glyceria* spp.) were also grouped together, i.e. floating sweetgrass (*G. fluitans*) and glaucous sweetgrass (*G. declinata*). The native red pondweed (*Potamogeton cheesemanii*) and the introduced curly pondweed (*P. crispus*) were grouped together, however, *P. cheesemanii* was predominately found. Of the water milfoils (*Myriophyllum* spp.), *M. propinquum* was most commonly found. Several semi-aquatic species from various genera, such as mints (*Mentha* spp.), forget-me-nots (*Myosotis* spp.), and docks (*Rumex* spp.) were also grouped together.

Statistical analysis

Two approaches were used in this study (Figure 3.1). The first analysis involved coarse-scale descriptions of microhabitat, which were calculated from the presence and dominance of aquatic plant taxa in quadrates (Figure 3.1a). Data collected from quadrates were also used to derive taxa richness, as well as, percentage cover of macrophyte patches and submerged fallen branches. The second analysis involved fine-scale descriptions and examined whether the proportional abundance of particular macrophyte species was useful in predicting *N. burrowsius* occurrence (Figure 3.1b). Forward step-wise discriminant function analyses were conducted for these two microhabitat description types in Statistica 6.0 (Statsoft Inc. 2001), using default tolerance settings. Discriminant function analysis is a technique used to build a predictive model of group membership, which in these analyses was the likelihood that microhabitat contained *N. burrowsius*. This method is computationally similar to multivariate analysis of variance (MANOVA), and has similar assumptions (Statsoft Inc. 2001), thus, proportional variables were arcsine square root transformed.

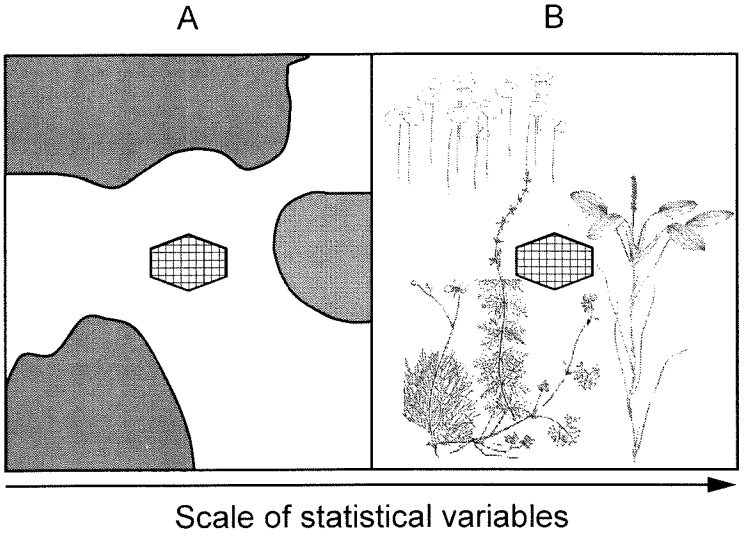

Figure 3.1. Conceptual diagram of the different scales of analysis used to quantify *N. burrowsius* microhabitat within one metre of Gee minnow traps. Hatched hexagons represent a Gee minnow trap placed within sampling location with (A) coarse-scale habitat descriptors such as, percentage macrophyte cover (indicated by shaded area), and (B) fine-scale details of the occurrence of each plant species.

Cross validation

Cross validation is an essential step in developing predictive models. The discriminant model based on macrophyte taxa was cross validated with 53 cases that were not included in generating the original model. These data were collected at St Andrews Drain and Dog Kennel Stream, and although the microhabitat was sampled at the same time using identical methods as above, large mesh Gee minnow traps had been used to capture *N. burrowsius*. These data were not included in generating the model due to concerns over the effectiveness of large mesh traps (Chapter 2). However, the data collected provided an opportunity to conduct preliminary tests of the validity and predictive ability of the discriminant model. Cross validation was conducted in Excel 10 (Microsoft Corporation 2002), using the classification functions obtained for the fine-scale discriminant model (Table 3.3).

RESULTS

In total, *N. burrowsius* were not caught in 30 of the 72 microhabitats sampled. The percentage of traps that did not capture *N. burrowsius* was similar at all sites, ranging from 39 – 44 %.

Coarse-scale descriptors using derived or estimated variables

Forward stepwise discriminant analysis of coarse-scale descriptors resulted in a significant model ($F_{(2, 69)} = 9.8$, $p < 0.001$, canonical correlation $R = 0.47$, Eigenvalue = 0.28). Two variables were included in the model indicating that *N. burrowsius* tended to occur in microhabitat with higher macrophyte taxa richness and/or with a higher percentage of submerged branches being present. Canonical coefficients indicated that macrophyte taxa richness and the percentage of submerged branches present provided approximately equal contributions to the predictive ability of the model (Table 3.1). Overall, this model correctly classified two thirds of cases included, however it classified microhabitat occupied by *N. burrowsius* more accurately (81 %) than microhabitat that they did not occupy (47 %).

Table 3.1. Standardised canonical coefficients for variables included in the coarse-scale forward step-wise discriminant analysis. Dashes indicate those variables not included in the model. Given are group means of variables relating to microhabitat in which *N. burrowsius* was absent or present.

Variable	Canonical coefficient	*N. burrowsius*	
		Absent	Present
Macrophyte taxon richness	0.98	1	2
% branches	0.96	0	3
% riparian vegetation	-	28	36
% macrophyte cover	-	58	65

Fine-scale descriptors using plant taxa groups

Seven macrophyte taxa or taxon groups were included in a statistically significant discriminant model ($F_{(7, 64)} = 4.2$, $p < 0.001$), in the fine scale investigation. Five of these included variables had a positive association with the occurrence of *N. burrowsius*, whereas, two macrophyte species were found to have a negative relationship (Table 3.2). This fine-scale discriminant model fitted the data better than coarse-scale descriptors, having a higher canonical correlation ($R = 0.56$), and an Eigenvalue of 0.45. Further, the classification functions correctly classified a greater number of cases used in the fine-scale model's development (Tables 3.3 and 3.4). Nonetheless, misclassification of cases, as both false positives and false negatives, occurred at all sites. Interestingly, the model correctly predicted *N. burrowsius* occurrence in microhabitat relatively accurately at the perennial St Andrews Drain (11 % incorrectly assigned), and Te Roto Repo o Tawera (17 % incorrectly assigned); whereas, approximately a third of cases were misclassified at the intermittent Dog Kennel Stream (28 % incorrectly assigned), and Hororata Spring (39 % incorrectly assigned). Furthermore, all of the cases representing traps placed in a willow bog habitat at Hororata Spring were misclassified. This was because traps captured *N. burrowsius* despite low macrophyte occurrence. This habitat is heavily shaded by several willow trees, which nonetheless, provide complex habitat structure via submerged roots and branches.

Table 3.2. Canonical discriminant functions standardised by within variances for the fine-scale discriminant analysis. Dashes indicate those taxa or taxon groupings not included in the model. Given are mean percentages of relative abundance at locations where *N. burrowsius* were present or absent.

	Coefficient	*N. burrowsius*	
		Present	Absent
Native aquatic taxa			
Lemna minor	0.90	46	25
Myriophyllum spp.	0.55	5	1
Potamogeton spp.	0.44	4	0
Azolla filiculoides	-	13	16
Wolffia australiana	-0.66	5	13
Introduced aquatic taxa			
Ranunculus spp.	0.45	8	0
Mimulus guttatus	0.37	5	0
Callitriche stagnalis	-0.23	0	1
Juncus articulatus	-	5	1
Glyceria spp.	-	26	25
Rorippa spp.	-	24	21
Semi-aquatic taxa	-	2	3

Table 3.3. Classification functions for the fine-scale macrophyte taxa discriminant model.

	N. burrowsius	
	Absent	Present
Constant	-1.21	-2.01
Lemna minor	0.91	2.62
Wolffia australiana	0.57	-1.48
Myriophyllum spp.	1.28	4.65
Potamogeton spp.	0.86	4.06
Ranunculus spp.	-0.20	2.12
Mimulus guttatus	0.99	3.44
Callitriche stagnalis	5.23	1.55

Table 3.4. Classification matrix of cases used to develop the fine-scale discriminant model.

		Predicted classification		
		N. burrowsius		
		Absent	Present	Percent correct
Observed classifications	Absent	25	5	83
	Present	12	30	71
	Total			76

Cross validation of macrophyte taxa model

The fine-scale macrophyte taxa discriminant model was cross validated using 53 cases not used to generate the original model. The classification functions for the discriminant model (Table 3.3), correctly classified all but one case where *N. burrowsius* were present (Table 3.5). However, there were a large number of false positives; i.e. microhabitats that the model classified as likely to contain *N. burrowsius*, yet none were actually caught. This is likely to be due to the low effectiveness of the large mesh Gee minnow traps used to obtain these data (Chapter 2).

Table 3.5. Classification matrix of cases used in the cross validation of the fine-scale discriminant model.

		Predicted classification *N. burrowsius*		
		Absent	Present	Percent correct
Observed classification	Absent	14	27	34
	Present	1	11	92
	Total			47

DISCUSSION

Characterising the association between fish species and their habitat is an important step in ecological rehabilitation, as small-scale habitat associations are often most important in determining limitations on fish abundance (Bond & Lake 2003). Based on qualitative assessments, Eldon (1993) summarised the general characteristics he considered described the optimal habitat for *N. burrowsius*. These were fluctuating levels of dissolved oxygen and water quantity, abundant in-stream vegetation, low water velocity (< 20 m/min), and the absence of other fish species. In this study I focused on quantifying the microhabitat of *N. burrowsius*, in particular the characteristics of vegetation. Comparisons of the two analyses conducted using independent variables of differing scales indicated that fine-scale variables

involving specific macrophyte taxa may provide the most robust means of characterising the habitat associations of *N. burrowsius*. Although both coarse- and fine-scale analyses were significant, Eigenvalues, and thus the fit of the discriminant model were higher for the fine-scale analysis. Furthermore, canonical correlation coefficients and the precision of the model in classifying correct cases were higher for fine-scale variables. Thus, certain aquatic macrophyte species were important predictors of the occurrence of *N. burrowsius*. Nonetheless, high macrophyte species richness, which is related to an increased probability of key macrophyte species being present, and the abundance of fallen branches, should also be considered. This was exemplified at Hororata Spring where the fine-scale model incorrectly predicted that no *N. burrowsius* would be present in a willow bog habitat that had few macrophytes present but contained a high abundance of fallen branches.

Although aquatic macrophyte richness was a significant predictor of *N. burrowsius* occurrence, a difference of only one species on average, distinguished the presence or absence of *N. burrowsius*. Nevertheless, this difference is likely to be important as many lowland waterways contain macrophyte communities which are largely monocultures, often of introduced species, such as *Rorippa* spp. (Howard-Williams et al. 1982). High macrophyte species richness may be positively related to *N. burrowsius* occurrence through association with habitats that have experienced fewer disturbances, including agricultural disturbance. This is because frequent disturbance often leads to communities containing a few tolerant species (Begon et al. 1990). An association between vegetation type and disturbance levels has also been shown for *N. diversus*, which were found at sites with low to moderate human impact (Hicks & Barrier 1996). The presence and complexity of aquatic plants can also influence the abundance and composition of macro-invertebrate communities colonising seasonal wetlands (Dudley 1988; De Szalay & Resh 2000). Further, high macrophyte diversity has been shown to be important in enhancing ecosystem functioning in wetlands (Engelhardt & Ritchie 2001).

The association of *N. burrowsius* with particular species of aquatic plants could have several explanations. *N. burrowsius* may have a direct preference for certain types of habitat structure provided by macrophyte species, because of the foraging opportunities and/or protection from predators they provide. Indeed, the growth form of aquatic vegetation can influence fish foraging and distribution. For example, Dionne & Folt (1991) in comparing plant density and architecture found that the type of plant species present was important in explaining variations in pumpkinseed sunfish (*Lepomis gibbosus*) foraging rates. Moreover,

N. burrowsius may prefer to spawn on particular macrophyte species (Eldon 1979c; Chapter 10), which may influence their general microhabitat use. Additionally, the high level of misclassification at sites where a predator was present (Hororata Spring and Dog Kennel Stream) could indicate subtly different patterns in habitat use by *N. burrowsius*, whereby, the presence of shortfin eels (*Anguilla australis*) could necessitate that *N. burrowsius* use microhabitat that provides greater cover. Indeed, predator avoidance behaviour has an important influence on habitat selection of prey (Kerfoot & Sih 1987). However, the association between *N. burrowsius* and macrophyte species could also be indirect, due to correlations with other factors, such as, hydrological fluctuations and water quality.

Seven macrophyte taxa or taxon groupings were included in the discriminant model. By examining characteristics of these species a greater understanding of the habitat associated with *N. burrowsius* may be gained. For example, Riis & Biggs (2001) categorised macrophyte species occurring in New Zealand streams and lakes on the basis of disturbance frequency and resource supply. Two introduced plant species (*R. trichophyllus* and starwort (*Callitriche stagnalis*)), and two native species (*M. propinquum* and *P. cheesemanii*) identified in my study were included in their habitat matrix model. These four species were all classified as having traits associated with high resource supply and high disturbance frequency (Riis & Biggs 2001). However, the species with the greatest canonical coefficient and thus contribution to the model was the native, small, free-floating macrophyte, duckweed (*Lemna minor*), which is commonly found in still pools, as it is easily removed by water flow. *Myriophyllum* and *Potamogeton* species were also often associated with pool areas of a habitat (personal observation). Thus, it appears that *N. burrowsius* were associated with low velocity pool areas, within productive and hydrologically disturbed sites. This result corroborates previous descriptions of *N. burrowsius* habitat (Skrzynski 1968; Eldon 1979a).

One native species was negatively associated with the presence of *N. burrowsius*. Water meal (*Wolffia australiana*), is the world's smallest flowering plant, typically being 1 x 1.5 mm (Johnson & Brooke 1998). This free-floating plant is often found in association with *L. minor* and Pacific azolla (*Azolla filiculoides*), as all three species require still water to persist (Coffey & Clayton 1988; Johnson & Brooke 1998). It is possible that the negative association with *W. australiana* is due to its effective smothering of the water surface because of its small size. This may reduce dissolved oxygen levels by reducing surface exchange with the atmosphere. A further explanation for this negative relationship is based on the differences in optimal pH for growth of these free-floating plants. McLay (1976) estimated that the optimum pH of *L.*

minor growth was pH 6.2, while that for *W. australiana* was pH 5.0. Thus, the presence of *W. australiana* is likely to indicate that the water is often relatively acidic. Other *Neochanna* species are more tolerant of acidic conditions (McDowall 1990). For example, Hicks & Barrier (1996) found *N. diversus* in habitats with a pH as low as 4.3, and Grainger (2000) found *N. apoda* in habitats that ranged in pH from 3.97 – 4.91. *N. burrowsius* may be more sensitive to low pH as it does not occupy peat wetlands as readily as other *Neochanna* species. Explanation of the negative association with *C. stagnalis* is less straightforward; however it is possibly related to the habitual occurrence of this macrophyte species in shallow areas (personal observation).

In conclusion, this study found that characterising *N. burrowsius* microhabitat by plant taxa did not increase the level of unexplained variation in statistical analysis; instead, it provided a more robust predictive model with practical applicability. Furthermore, this study indicates that methods of wetland monitoring that are based on detailed vegetation plots, such as Clarkson et al. (2004), are preferable to coarse-scale or generalised characterisation of habitat. The identification of particular plant species associated with the occurrence of *N. burrowsius* provides an important insight into its habitat requirements, irrespective of whether the mechanism behind this association is identified. Thus, the findings of my study have implications for the effective characterisation of *N. burrowsius*' remaining habitat, monitoring of habitat condition, guiding rehabilitation and management actions, and in identifying potential translocation sites.

Chapter 4.

Assessing the security of *Neochanna burrowsius* populations.

INTRODUCTION

An increasingly important objective in conservation ecology is determining the security of species and their populations against extinction (Belovsky et al. 1999; Benton 2003; Cardillo 2003). This necessitates developing methods of assessing the vulnerability of a species and monitoring relevant attributes of populations. However, extinction can occur via a multiplicity of contributing factors, or extreme events, on large or local scales. Further, as studies of Arctic charr (*Salvelinus alpinus*) in western Irish lakes have indicated, mechanisms of local extinction can be difficult to determine once a species has been lost (Igoe et al. 2001). Conversely, substantial freshwater biodiversity can persist in highly degraded urban areas (Dudgeon 2003). These examples highlight the need for a greater understanding of the factors involved in the persistence and extinction of species. Nonetheless, it is recognised that intrinsic species traits, as well as extrinsic events can strongly influence the security and persistence of species.

Comparisons within phylogenetic groupings have consistently indicated that particular intrinsic species traits are related to an increased likelihood of extinction, including the level of ecological specialisation, body size, fecundity, movement patterns, and distribution (Angermeier 1995; Fagan et al. 2001; Dulvy & Renyolds 2002; Cardillo 2003; Jones et al. 2003; Koh et al. 2004). Various mechanisms have been suggested to explain these correlations. Specialisation may restrict the ability of a species to adapt to environmental change, compared with more generalist species. Additionally, large body size in comparison with other similar species is correlated with life-history characteristics, such as low fecundity and delayed reproductive maturity. Thus, reproductive traits may explain much of the relationship between body size and extinction vulnerability (Bennett & Owens 1997; Fagan et al. 2001; Cardillo 2003; Jones et al. 2003). Undeniably, reproductive ability is important and traits that improve recruitment success are likely to improve persistence. For example, the lack of parental care in species of North American minnow (Cyprinidae) increased the

likelihood that they were threatened, compared with specialised nest building species (Johnston 1999). A restricted distribution, where extreme stochastic events could affect a large proportion of the area occupied by a species may also greatly increase the likelihood of a species' extinction (Jones et al. 2003). For example, Angermeier (1995) examined 197 species of freshwater fish in Virginia and found that limited physiographic range and occurrence in waterbodies with a limited size range increased the likelihood of extinction. A further species trait increasing vulnerability to extinction is the need to migrate between separate habitats to complete a life cycle, e.g. diadromy in some fishes (Angermeier 1995).

Within a species it is important to consider the abundance, size and spatial distribution of populations, as well as small scale population dynamics, when determining population persistence, especially in fragmented landscapes (Baguette & Schtickzelle 2003). Habitat loss and fragmentation, often driven by anthropogenic factors, may increase the risk of extinction (Fahrig 2001; Fagan et al. 2002). In such situations the carrying capacity of the remaining habitat is an important determinant of extinction likelihood (Belovsky et al. 1999; Hilderbrand 2003). This is because a low carrying capacity limits the population size attainable and low population size increases the probability of population extinction through random declines in survival and birth rates, i.e. demographic stochasticity (Belovsky et al. 1999). Furthermore, as habitat is removed or its quality reduced, individuals may be concentrated into ever smaller patches of tolerable habitat, a process that can result in abundance fluctuations within subpopulations and is linked to local extinctions (den Boer 1981; Belovsky et al. 1999; Crooks et al. 2001).

Populations can also become extinct due to environmental stochasticity, i.e. deleterious environmental conditions and periodic catastrophes (Raimodo et al. 2004). Increased disturbance can potentially lead to large fluctuations in population size, increasing the likelihood of the stochastic extinction of populations (Karr 1982). Furthermore, how populations fluctuate in relation to each other is important. Specifically, independent population dynamics increases persistence, and synchronicity increases extinction likelihood (den Boer 1981; Raimodo et al. 2004). Nevertheless, high levels of local population extinction may not result in extinction of a species. If the level of dispersal is high, leading to frequent colonisation events, species persistence may occur via meta-population dynamics (Hanski & Simberloff 1997).

Collectively, consideration of these extrinsic and intrinsic factors may indicate the vulnerability of a species and its populations. Canterbury mudfish (*Neochanna burrowsius*), the focus of this study, has a relatively small body size, reproduces in its first year, and has high fecundity compared with other *Neochanna* species (Cadwallader 1973; Eldon 1979c). Further, *N. burrowsius* is non-migratory, tolerant of a wide range of environmental conditions, and is capable of dispersing and recolonising habitat after local extinction (Eldon et al. 1978; Eldon 1979a; Meredith 1985). These characteristics are likely to increase the species chances of persistence. However, *N. burrowsius* also has traits that are likely to increase its vulnerability to extinction, such as lack of parental care, specialisation to wetland habitat, low genetic diversity, and its restriction to the Canterbury Plains, of South Island, New Zealand (Eldon 1979c; McDowall 1997a, 1998; Davey et al. 2003). Furthermore, its wetland habitat has experienced extensive modification and fragmentation over the last 150 years (McDowall 1998). This has involved mainly wetland drainage, which has increased the intensity and frequency of drought on the Canterbury Plains (Sagar & Jellyman 1985). Such increased levels of disturbance are likely to increase the risk of stochastic extinction. Indeed, local extinctions of *N. burrowsius* have been documented (Skrzynski 1968; Cadwallader 1973; Eldon 1979a, 1993). Currently, *N. burrowsius* habitats are surrounded by highly productive agricultural land and consequently, are subject to disturbance by waterway management practices and the presence of stock (Hudson & Harding 2004). In such situations, it is important to be able to objectively identify populations at risk.

An objective of the New Zealand *Neochanna* recovery plan (DoC 2003, p. 15) states that 'annual monitoring of at least three populations within each species evolutionary significant unit will provide an accurate assessment of how "secure" each species is.' In this chapter I examined several common measures used to characterise populations that are likely to be useful in assessing population security. These were, estimated population size and catch per unit effort (CPUE), as well as levels of aggregation and abundance fluctuations within each site. Further, I briefly examined factors likely to be adversely affecting populations. I hypothesised that populations subject to factors known to affect population security deleteriously, such as habitat removal, would exhibit particular characteristics, such as small population size, declining CPUE, high levels of aggregation, and large fluctuations in abundance.

METHODS

Sampling design

The design of this study involved sampling *N. burrowsius* in multiple habitats within a site at a scale of approximately 10 – 100 m, covering most of the distribution of a population (Figure 4.1). Locations within each of four study sites were sampled during austral autumns from 1999 – 2002. Within each site, four to seven distinct habitats were selected based on hydrological separation and/or differences in habitat morphology. See Chapter 1 for descriptions of sampled habitats within sites.

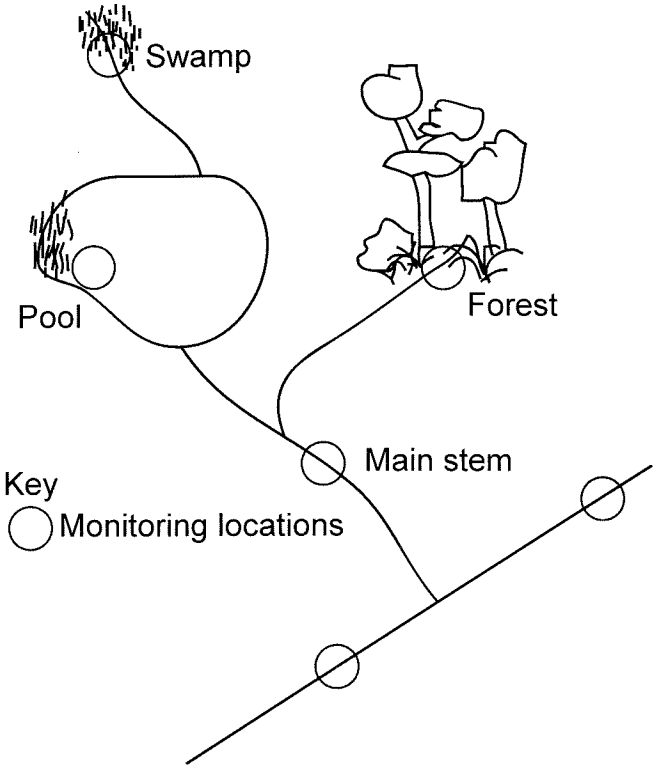

Figure 4.1. Hypothetical example of *N. burrowsius* habitat survey design used in this chapter, with distinctive habitat types sampled within each site.

Fish capture

N. burrowsius abundance was determined using different sampling methods in each year, as more effective techniques were progressively developed (Chapter 2). Field surveys conducted during 1999 and 2000 used Gee minnow traps (GMT) only. Subsequent surveys during 2001 and 2002 used a combination of Gee minnow trapping and electrofishing (EFM) techniques. Sampling in 2002 was the most intensive, being conducted twice in autumn (April and May). During 2002, the same number of GMT were used within each habitat on

each sampling occasion, and electrofishing was standardised by maintaining the same unit effort, recorded as the number of minutes electric current was passed through the water, between sampling occasions. All fish captured were anesthetised, measured, and re-released into their previous habitat (see Chapter 2 for fish handling methods).

Population size estimates

The population size at each of four sites was estimated in autumn 2002 using mark-recapture methods, with a single period of recapture one month after release. Thus, during April 2002, *N. burrowsius* were batch tagged by injecting a small amount of non-toxic acrylic hobby paint (FolkArt®, Plaid Enterprises Inc., GA, USA), into the fleshy caudal peduncle region.

The Petersen mark-recapture method was used to estimate the number of *N. burrowsius* present at each site calculated using the Bailey correction (Krebs 1998). Due to low recapture rates, a Poisson approximation was used to calculate 95 % confidence intervals. The main assumption of the Peterson method is that the system is closed. A closed population does not change in size as a result of births, deaths, or movements, during the period of study (Krebs 1998). Although each *N. burrowsius* sampling location within a site was unlikely to be closed, the population size within each site was not likely to have changed substantially over one month. Thus, *N. burrowsius* populations were assumed to be closed at the site level. The results for each sampling location within a site were therefore pooled to estimate the population size at the site level. Eldon et al. (1978) concluded that one month was insufficient for effective mixing of marked *N. burrowsius*. However, in the present study a substantial flood event occurred two weeks prior to the re-sampling period in May 2002. This is likely to have sufficiently mixed the fish as recapture rates were low. Initial attempts at population estimation, involving longer periods between mark and recapture, resulted in even lower recapture rates.

Aggregation and abundance fluctuations

Relative abundance during each sampling period was calculated as the proportion of *N. burrowsius* caught in each habitat within each site, corrected for the area sampled. However, at Hororata Spring, area was not included in 2001 as most fish were found in a small subterranean refuge (Chapter 7), which skewed density calculations. The use of a proportional variable allowed the comparison of *N. burrowsius* abundance determined by differing GMT and EFM sampling methods during each sampling period. Density ($\log_{10}$

transformed) of *N. burrowsius* in pools in the presence or absence of shortfin eel (*Anguilla australis*) were compared using ANOVA.

To compare the level of aggregation in *N. burrowsius* within each site, Morisita's Index was used. This index was calculated from the number of fish caught in each habitat during each sampling period, following the equation given by Hurlbert (1990). Morisita Index values greater than one indicate that a distribution is aggregated. The co-efficient of variation in *N. burrowsius* abundance within each habitat at each sampling occasion was also calculated to compare fluctuations in abundance. Differences in these measures between sites were tested using ANOVA.

RESULTS

Population size estimates

During an eight day period in April 2002 a total of 849 *N. burrowsius* were caught and batch tagged at the four sites (Te Roto Repo o Tawera: n = 295, Hororata Spring: 67, St Andrews Drain: 391, Dog Kennel Stream: 96). Repeat sampling one month later caught 1120 *N. burrowsius* in total, however tag recovery in some sites was low (Table 4.1). Te Roto Repo o Tawera was estimated to have the largest population, whereas Hororata Spring was the lowest (Table 4.1). Confidence limits were wide for Hororata Spring and Dog Kennel Stream due to the low number of marked fish recaptured (Figure 4.2).

Table 4.1. Estimates of *N. burrowsius* population sizes at the four study sites during autumn 2002, calculated using the Peterson mark-recapture method.

Site	Number tagged	Percentage tag recovery	Petersen estimate
Tawera	295	14	3086
Hororata	67	3	1359
St Andrews	391	25	1797
Dog Kennel	96	8	2359

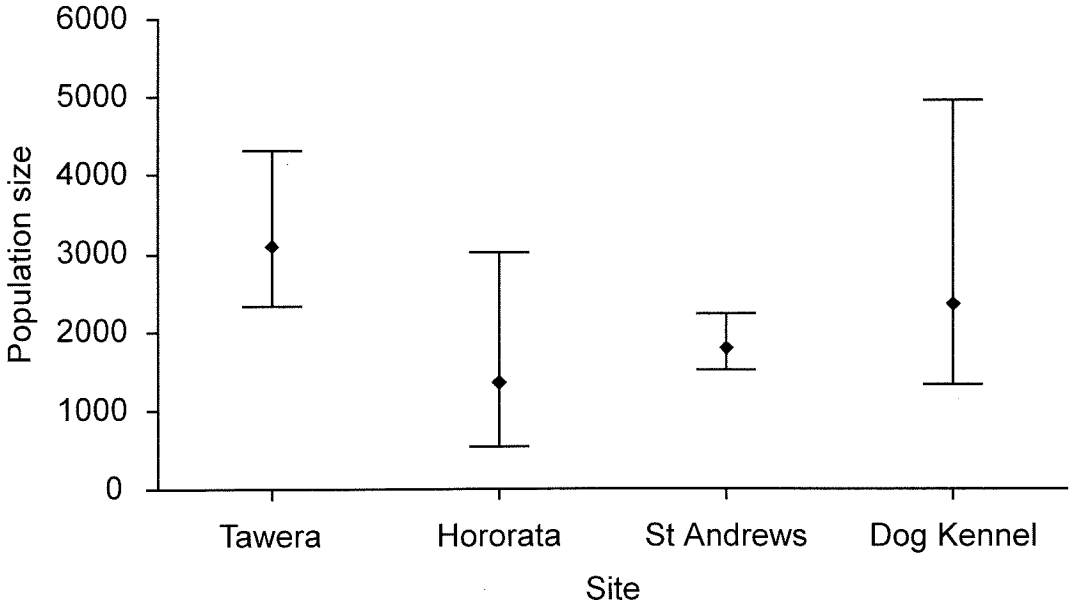

Figure 4.2. Estimates of mean *N. burrowsius* population size at the four study sites from Petersen estimates with Bailey correction factors. 95% confidence intervals, estimated assuming a Poisson distribution are shown.

Population trends

Available CPUE data for each site indicated that Te Roto Repo o Tawera and St Andrews Drain had higher values than the other two sites in 2002 (Table 4.2). Trends in abundance were variable, but could conceivably indicate that within Te Roto Repo o Tawera *N. burrowsius* abundances were increasing and that in Dog Kennel Stream *N. burrowsius* abundances were recovering after a reduction during 2000 and 2001. In comparison, within St Andrews Drain and Hororata Spring the abundance of *N. burrowsius* may have declined in 2002. However, it is difficult to draw any firm conclusions from this limited time series as trends were not strong or consistent.

Table 4.2. *N. burrowsius* catch per unit effort (CPUE) from Gee minnow traps only, for the four sites studied during the period 1999 – 2002. Previous qualitative abundance assessments for sites are given, sourced from the New Zealand Freshwater Fish Database (NZFFD; McDowall & Richardson 1983). Dashes indicate that Gee minnow trapping was not conducted.

Period	Site			
	Tawera	Hororata	St Andrews	Dog Kennel
1975 – 1982	Common[1]	Common[2]	Abundant[3]	Abundant[4]
1999	3.3	2.6	16.6	2.1
2000	-	-	-	0.6
2001	2.3	-	27.2	0.6
April 2002	10.2	0.1	15.8	1.4

[1] NZFFD card number 3675

[2] NZFFD card number 122

[3] NZFFD card number 50715

[4] NZFFD card number 50740

Patterns in relative abundance

The distribution of *N. burrowsius* within sites was patchy. Relative abundance, measured as the proportion of *N. burrowsius* caught in each habitat, during each sampling period, differed between habitats and varied spatially between sampling periods. Further, the level of tag recapture differed between habitats within each site. These patterns were related to the presence of site-dependent factors.

Te Roto Repo o Tawera

Te Roto Repo o Tawera was a hydrologically stable site with perennial flow and low agricultural disturbance. At Te Roto Repo o Tawera *N. burrowsius* patterns in abundance throughout the site did not change substantially, either between years or over one month (Figure 4.3). *N. burrowsius* were predominantly captured in a series of thin deep pools in the Tributary habitat. The percentage of recaptured, tagged *N. burrowsius* was also higher in this habitat compared with those on the mainstem of the waterway (Table 4.3).

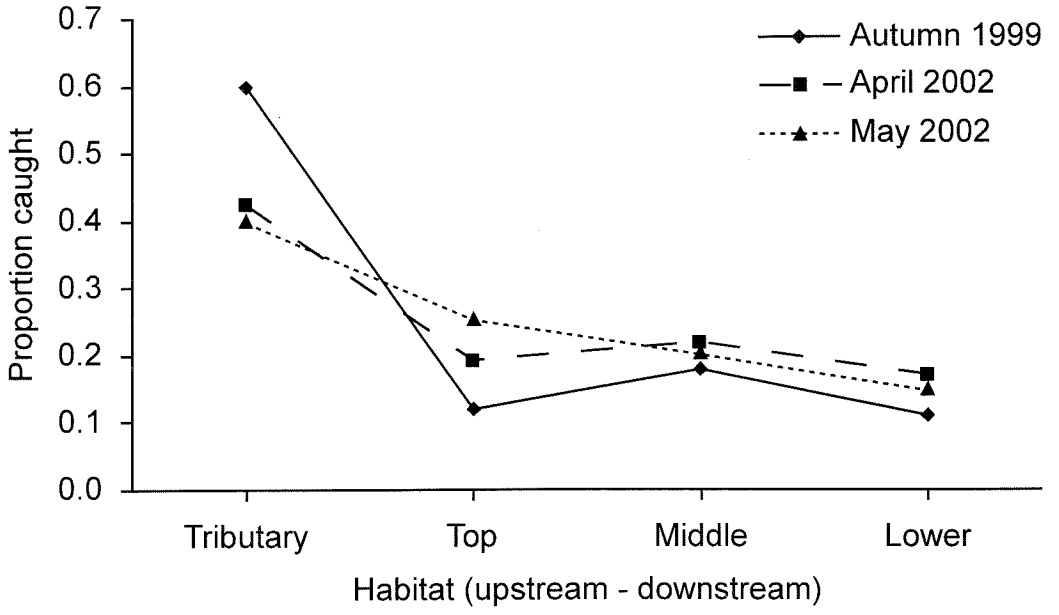

Figure 4.3. The proportion of the total *N. burrowsius* caught in each habitat at Te Roto Repo o Tawera during autumn 1999 and 2002. Top, Middle and Lower habitats were on the main waterway. In 1999, 60 *N. burrowsius* were caught in total, whereas in 2002 295 were caught in April, and 424 in May.

Table 4.3. The number of *N. burrowsius* caught and tagged in April 2002, and the percentage subsequently recaptured during May 2002 in habitats within Te Roto Repo o Tawera.

N. burrowsius	Habitat			
	Tributary	Top	Middle	Lower
Captured and marked	175	42	42	36
Percent recaptured	18.3	9.5	4.8	8.3

Hororata Spring

At Hororata Spring large differences in water level were observed between years during the autumn months and the distribution of *N. burrowsius* throughout the waterway differed, concurrently. *N. burrowsius* were more aggregated in upstream habitats during dry years but congregated in downstream shallow wetland habitat during wetter years (Figure 4.4). In the severely dry year of 2001, the majority of fish were found emersed, at approximately 50 cm depth within the substratum of the Confluence habitat (Chapter 7). However, very few *N.*

burrowsius had previously been found at this location and *N. burrowsius* were also sparse in this habitat in the subsequent wet year. Hororata Spring dried from downstream, contracting to deep pools near the source of the springs, which retained water even during extreme drought events. During the wettest sampling period of 2002, upland bully (*Gobiomorphus breviceps*) and *A. australis* largely remained in upstream habitats that contained permanent water (Table 4.4). In contrast, the scarcity of *N. burrowsius* in these upstream habitats, with aggregation in the downstream Wetland habitat instead, possibly indicates avoidance of predation and competition. Thus, the distribution of *N. burrowsius* within this site may be constrained by water fluctuations and inter-specific interactions.

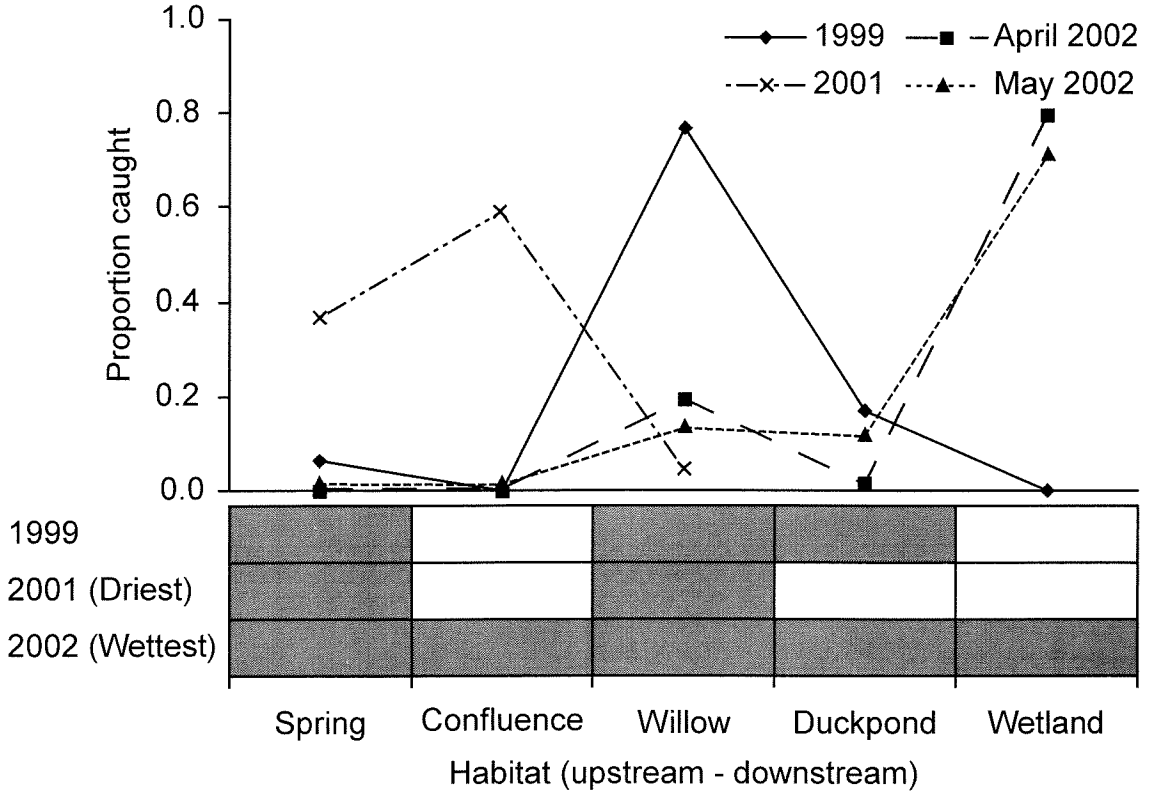

Figure 4.4. The proportion of the total number of *N. burrowsius* caught in each habitat at Hororata Spring over three years in which differing hydraulic conditions were experienced. Rectangles indicate whether water was present (grey) or absent (white) in each habitat at the time of sampling. In 1999, 47 *N. burrowsius* were caught in total; in 2001, 176 were found; and in 2002, 67 were captured in April and 59 in May.

Table 4.4. Abundance of *N. burrowsius*, *G. breviceps*, and *A. australis* throughout Hororata Spring during April and May 2002. Mean numbers of *G. breviceps* and *A. australis* captured in each habitat are derived from both Gee minnow trapping and electrofishing.

Habitat	Species		
	N. burrowsius	*G. breviceps*	*A. australis*
Spring Pool	0.5	6	3.5
Confluence	0.5	13	0.5
Willow Pool	10.4	12	1
Duck Pond	4	6	1
Wetland	47.5	2	1.5

St Andrews Drain

St Andrews Drain was a small largely channelised, perennially flowing waterway. In 1999, 80 % of *N. burrowsius* caught were within the Pool habitat, rather than in adjoining channelised habitats (Figure 4.5). Following the introduction of beef cattle to the property in 2002 the proportion of *N. burrowsius* caught in the Pool dropped. Instead fish were congregated immediately downstream, in the Ditch habitat, below a culvert that forms the Pool (See Chapter 1). This pattern of aggregation was similar during both April and May 2002 (Figure 4.5). This shift in *N. burrowsius* distribution between 1999 and 2002 was likely due to habitat degradation by cattle, as the Pool area was easily accessible. Comparison of photos taken before (Figure 4.6a), and after (Figure 4.6b) the presence of cattle clearly shows the removal of in-stream vegetation and the disturbance of sediment in the Pool area. Waterway sections up- and down-stream of the Pool were not as heavily grazed due to the presence of steep banks and fences restricting access. Recapture of tagged *N. burrowsius* was highest in the Ditch habitat, although the Pool also had an above average level of recapture (Table 4.5).

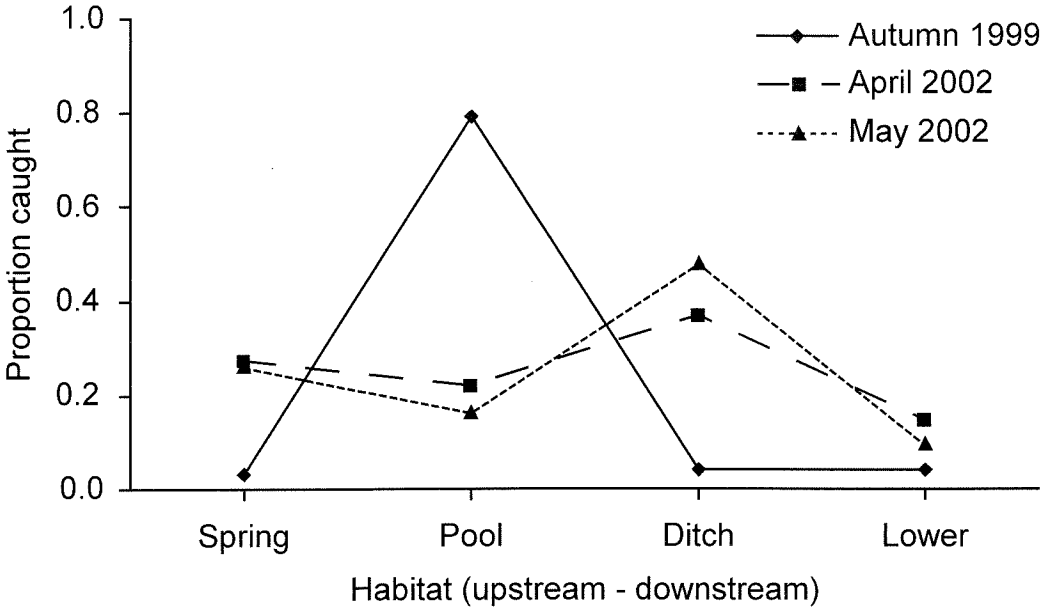

Figure 4.5. The proportion of total *N. burrowsius* caught in each habitat at St Andrews Drain. In 1999 74 *N. burrowsius* were caught in total, whereas in 2002, 391 and 409 were caught in April and May respectively.

Table 4.5. Numbers of *N. burrowsius* caught and tagged, and the percentage subsequently recaptured in habitats within St Andrews Drain in autumn 2002.

N. burrowsius	Habitat			
	Spring	Pool	Ditch	Lower
Captured and marked	72	110	154	55
Percent recaptured	15.3	28.2	32.5	5.5

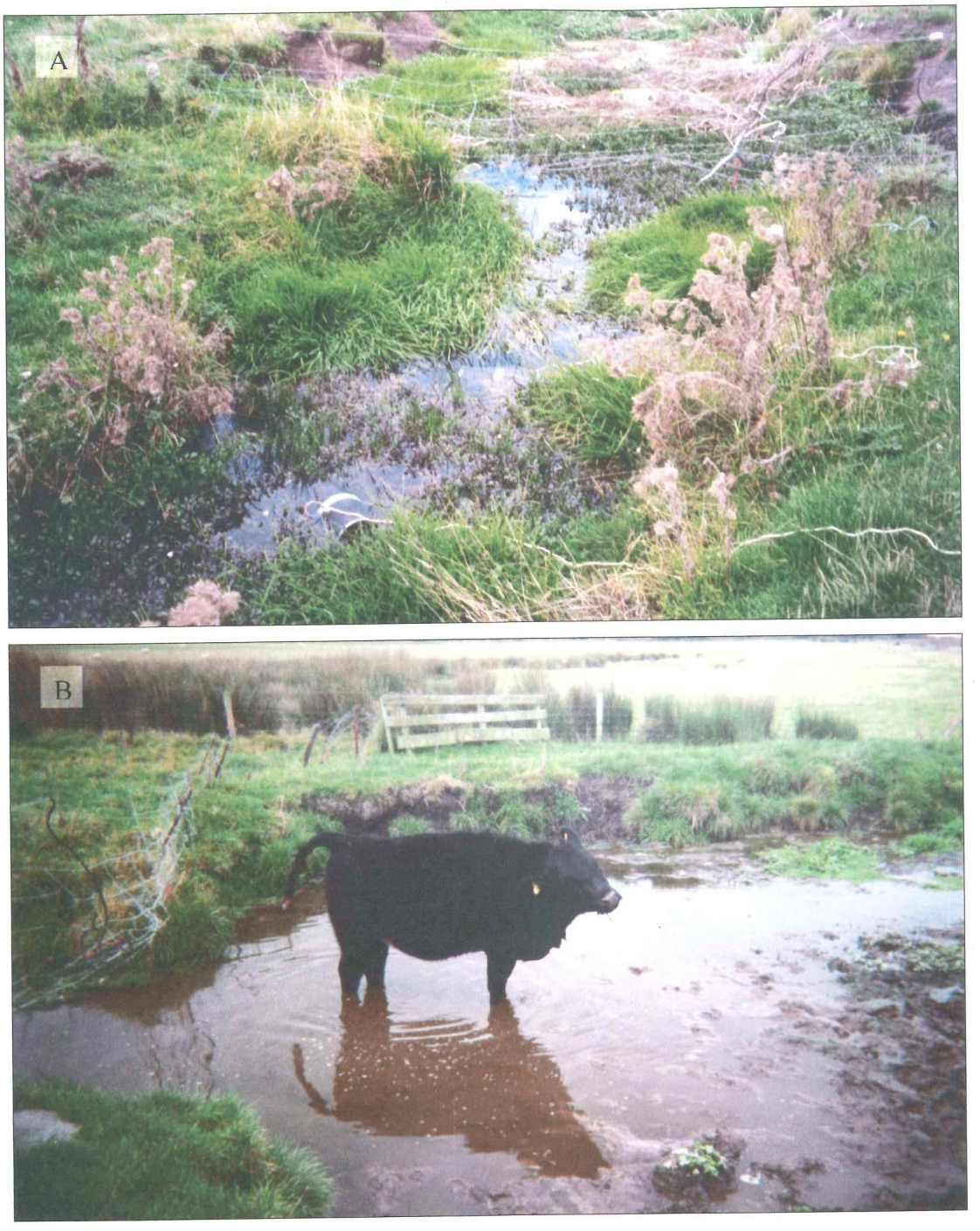

Figure 4.6. The Pool habitat at St Andrews Drain in 1999 (A), and 2002 (B), before and after the introduction of beef cattle to the property. Note that it is the same fence at top of photo A, and to the left in photo B.

Dog Kennel Stream

The pools at Dog Kennel Stream were relatively similar in size and visual appearance; however, *N. burrowsius* were often aggregated in particular pools. Furthermore, the

distribution of *N. burrowsius* throughout this series of pools differed substantially over the long and short term (Figure 4.7). In 1999 *N. burrowsius* were aggregated in Pools 1 and 6, whereas in April 2002, most *N. burrowsius* were captured in Pool 2, despite no *N. burrowsius* previously being captured in this pool. Periods of drought affected the pools differently, with deeper pools, and those still receiving groundwater inflow retaining larger volumes of water for longer periods. This was evident in 1999 when Pool 2 was calculated as only being 8 % full of water, providing possible explanation as to why no fish were caught there. Yet, water level alone does not fully explain the patterns observed as few *N. burrowsius* were caught in Pools 3 and 4 despite these being approximately 100 % full in 1999. Whereas, Pools 1 and 6, in which most fish were captured in 1999 were 24 % and 42 % full, respectively.

A further extrinsic factor likely determining patterns of *N. burrowsius* aggregation was the presence of *A. australis*, which were found to have a negative effect on the abundance of *N. burrowsius* caught in pools ($F_{(1,13)} = 5.1$, $p = 0.04$). *A. australis* were not sampled in 1999, however, in 2001 one, and in 2002 three *A. australis*, were caught and removed from the site. It is likely that *A. australis* invasion into the site was high in 2002 because movement between the pools was less hindered due to higher flows.

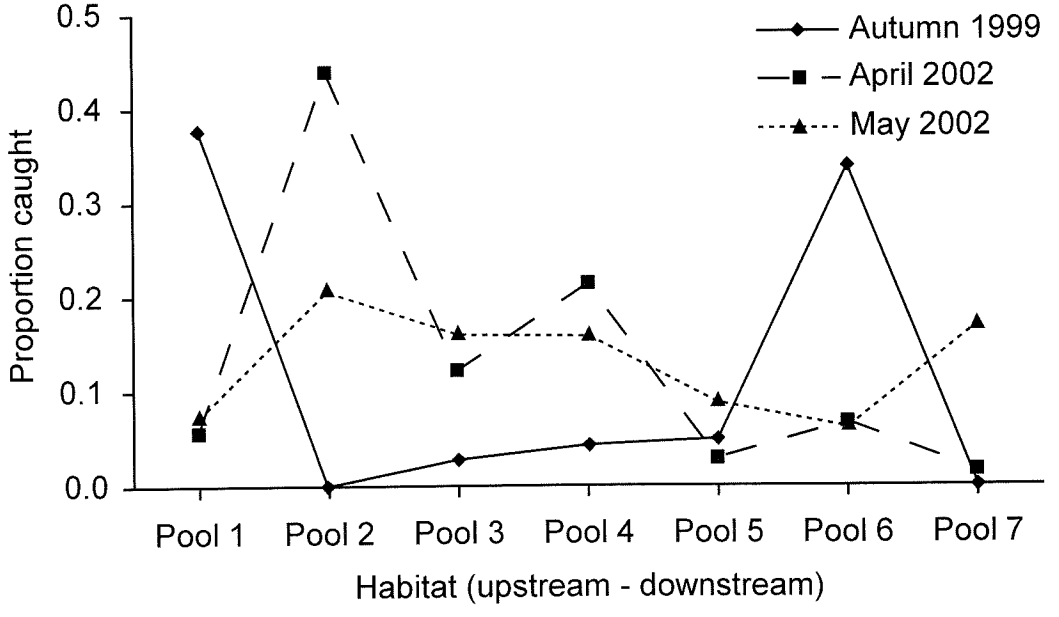

Figure 4.7. The proportion of total *N. burrowsius* caught in each pool habitat at Dog Kennel Stream. In 1999, 47 *N. burrowsius* were caught in total; whereas in 2002, 96 were caught in April, and 128 in May.

Table 4.6. Numbers of *N. burrowsius* caught and tagged in April 2002, and the percentage subsequently recaptured in May 2002 in pool habitats within Dog Kennel Stream.

N. burrowsius	Pool						
	1	2	3	4	5	6	7
Captured	6	37	15	17	5	5	1
Percent recaptured	0	2.7	6.7	0	0	40	0

Aggregation within sites

All sites had average Morisita Index values greater than one, indicating that the distribution of *N. burrowsius* was aggregated within each site (Figure 4.8). This result confers with patterns of relative density between habitats at each site presented in the preceding section. Further, there were significant differences in Morisita Index values between sites ($F_{(3,9)} = 4.4$, $p < 0.04$), mainly due to the high level of aggregation observed at Hororata Spring. At this site most *N. burrowsius* appeared to be aggregated in a single habitat during each sampling period.

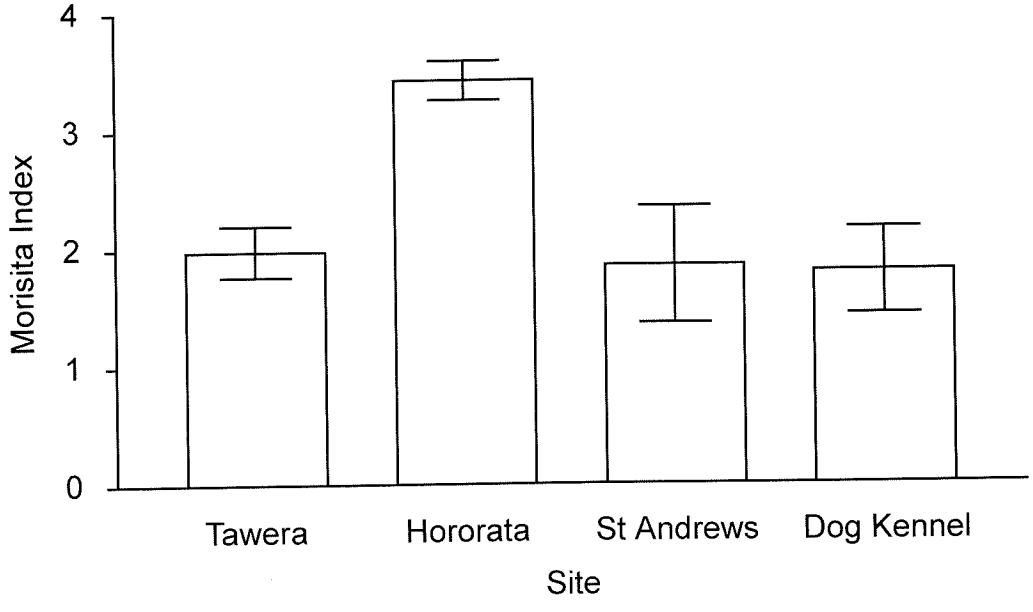

Figure 4.8. Mean Morisita Index values (± 1 SE) calculated using *N. burrowsius* abundance for each site during each sampling period.

Abundance fluctuation within habitats

Not only were *N. burrowsius* aggregated in particular habitats within sites, but also the location of aggregations differed between sampling periods at some sites. Thus, within each habitat, the proportion of *N. burrowsius* present fluctuated, greatly. To compare the extent of abundance fluctuations within habitats, between sites, the coefficient of variation for *N. burrowsius* abundance was calculated for each habitat between sampling periods. Significant differences in the level of variation between sites occurred ($F_{(3, 16)} = 7.6$, $p = 0.002$), with Hororata Spring and Dog Kennel Stream having the highest variation in abundance within each habitat (Figure 4.9). In contrast, the level of fluctuation at Te Roto Repo o Tawera was low, as depicted in Figure 4.3. Thus, the absence of extrinsic factors likely to affect *N. burrowsius* was reflected in low levels of abundance fluctuation at this site.

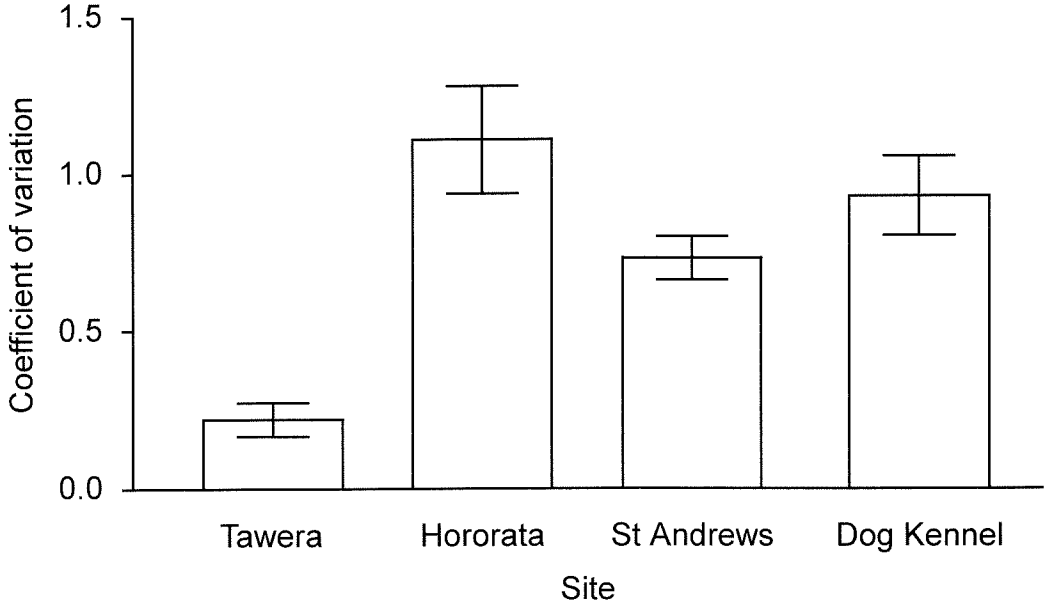

Figure 4.9. Mean coefficient of variation (± 1 SE) in *N. burrowsius* abundance for each site calculated as the coefficient of variation for each habitat across the three sampling periods.

DISCUSSION

Techniques of simulation modelling, such as population viability analysis (PVA) using intrinsic species traits, have been forwarded as a valuable management tool to determine population security (Boyce 1992). However, they are severely limited by the availability of sufficiently accurate data, often cannot be reliably used to determine population trends, and have been widely criticised (Hamilton & Moller 1995; Ludwig 1999; Fieberg & Ellner 2000). Moreover, these models are only applicable to a certain set of circumstances and species traits. Many PVA models perform poorly when dealing with species with high fecundity, short life-spans, and high density populations. Thus, these models are largely not applicable to many fish species, including *N. burrowsius*. In this study I examined several common population characteristics to evaluate their usefulness in identifying populations with a reduced security of persistence. Collectively, I found that similar results were obtained from each measure. Thus, estimated *N. burrowsius* population size, total CPUE and the stability of distribution and abundance patterns were higher for Te Roto Repo o Tawera and lower for Hororata Spring. Qualitative evaluation of the four *N. burrowsius* sites studied indicated that the population at Te Roto Repo o Tawera was likely to be the most secure, as the site was hydrologically stable and largely unmodified. In comparison, at Hororata Spring frequent habitat drying occurred, both *A. australis* and *G. breviceps* were present, and stock damage was evident. These are all factors that are likely to reduce population security. However, despite the sampled population measures corroborating one another, the amount of information and the level of certainty provided by each measure in determining population security differed.

Population size

The population sizes found in this study are comparable with previous estimates obtained for *N. burrowsius*. Eldon et al. (1978) used several methods to estimate the population size at an extensive site at Clearwell, Mid Canterbury, calculating that approximately 2000 – 4000 *N. burrowsius* were likely to be present. Nonetheless, the 95 % confidence limits of the estimates in my study indicated that the *N. burrowsius* population present at Hororata Spring may comprise 500 – 3000 individuals, while at Dog Kennel Stream 1300 – 4900. Such wide confidence intervals, influenced by low recapture rates are an obstacle to determining conservation status based on population size alone. This is often unavoidable because as species become increasingly rare, or difficult to capture, resulting estimates of the numbers left becoming increasingly less accurate (Green & Young 1993; Oborny et al. 2005).

Considering the low number of fish captured using both active and passive methods at Hororata Spring (Chapter 2), it is likely that the true *N. burrowsius* population size falls toward the lower end of these estimates. The same may hold for Dog Kennel Stream. However, the extent of overlap in the estimates for each site means it is difficult to confidently rank the security of each population. It is also recognised that estimates of population size are fraught with methodological difficulties and assumptions that are difficult to uphold (Krebs 1994). Thus, population estimates should be used as an estimate of population security with some caution.

It is also unclear whether population size relates directly to extinction likelihood in *N. burrowsius*, because having a large population size may not necessarily guarantee population security. For example, the large population at Clearwell, Mid Canterbury is now extinct 'owing to the illegal realignment of a watercourse on another property' (Eldon 1993, p. 8). Other *N. burrowsius* population extinction events have been attributed to direct habitat destruction, hydrological modification, and the introduction of piscivorous fish (Eldon 1979a; McDowall 1990; DoC 2003). Thus, it is likely that extrinsic factors, such as agricultural activities and increasing drought frequency are more important than intrinsic population characteristics, such as size, when considering *N. burrowsius* population security. This conclusion is reinforced by Duncan & Lockwood (2001) who considered that due to the level of human alteration on freshwater systems, extrinsic factors may overwhelm intrinsic traits, thus increasing the vulnerability of otherwise resilient species. Accordingly, it is important to consider extrinsic factors, as well as intrinsic species traits in determining vulnerability to extinction (Cardillo 2003).

A further consideration is that in certain circumstances the actual population size may not reflect the 'genetic effective population size', and in fact it rarely does so in natural populations (Dulvy et al. 2004). The concept of genetic effective population size is based on the likelihood of unique genes being passed on to the next generation. Various factors may reduce the diversity of genes maintained within a population, and thus effective population size, including unequal sex ratios, dominance hierarchies, and fluctuating population size (Dulvy et al. 2004; Jacobsen et al. 2005). *N. burrowsius* exhibit characteristics such as, fluctuating population sizes and the possible occurrence of previous population bottlenecks (Eldon 1979a; Davey et al. 2003). Moreover, low genetic diversity is considered an indication of low effective population size (Jacobsen et al. 2005), and in some cases *N. burrowsius* populations have extremely low genetic diversity (Davey et al. 2003). Thus,

although the population sizes of *N. burrowsius* estimated from mark-recapture methods appear high, the effective population size is likely to be much lower because of the low number of unique genes likely to be present. The consequence of having a low effective population size is that many more individuals are required to maintain genetic diversity within a population (Jacobsen et al. 2005).

Trends in CPUE

Studies have emphasised that not only small population size, but also low density is an indication of extinction vulnerability (Oborny et al. 2005). Examination of available temporal data of *N. burrowsius* density for each site, measured as total CPUE obtained from Gee minnow traps, leads to the conclusion that CPUE data would need to be collected over a longer time period before a valid trend could be determined. Restricted temporal scope is a common impediment to determining a trend in abundance with any certainty (Shea & Mangel 2001). In saying this however, it is appreciated that robust and reliable predictions of long-term extinction probabilities may require unattainable amounts of data (Fieberg & Ellner 2000). Thus, it is unlikely that a single CPUE value for a population will be sufficient to assess population security even with a large data set. Additionally, catchability must be constant for CPUE to form a useful index of abundance (Rose & Kulka 1999). A constant relationship between CPUE and abundance in *Neochanna* may not always hold. For example, Barrier et al. (1996) suggested that higher CPUE results for black mudfish (*Neochanna diversus*) occurred during rainy nights. Further, the effectiveness of Gee minnow traps in capturing *N. burrowsius* was reduced in the presence of *A. australis*, and altered by habitat characteristics (Chapter 2). The importance of this issue is demonstrated in the cautionary example of the collapse of the Atlantic cod (*Gadus morhua*) fishery. Reliance on the assumption that CPUE increased linearly with abundance lead to population sizes being grossly overestimated. This was because aggregation behaviour in *G. morhua* meant that density, and thus CPUE was high in areas sampled, despite declining overall abundance (Rose & Kulka 1999). Thus, it has been emphasised that it is important to include spatial information when evaluating extinction likelihood (Rose & Kulka 1999; Oborny et al. 2005).

Aggregation and abundance fluctuation within sites

Organisms may aggregate in particular locations due to its high resource value compared with the surrounding habitat (Begon et al. 1990; Essington & Kitchell 1999; Holbrook et al. 2000). Thus, in this study, low levels of aggregation were assumed to indicate the likelihood that all habitats were uniformly suitable. Whereas, high levels of aggregation were assumed to

indicate that suitable habitat was limited and individuals congregated within it, rather than occupying surrounding, less suitable habitats. Aggregation was evident at each *N. burrowsius* site studied, although it was greatest at Hororata Spring, where hydrological fluctuations and inter-specific interactions likely affected habitat suitability. Thus, the distribution of *N. burrowsius* was patchy and although some sites were extensive, not all available habitats sustained similar numbers of *N. burrowsius*.

The percentage of recaptured, tagged fish may be informative as a relative measure of the turnover rates for occupancy of a particular habitat, and thus its suitability. High turnover rates, i.e. low percentage of recapture, have been associated with high levels of movement (Rodríguez 2002), and hydrological regime (Albanese et al. 2004), specifically perennial and intermittent flows, with recapture rates being lowest in highly fluctuating sites (Dunn 2003). Similarly, intermittent *N. burrowsius* sites showed lower percentage recapture than perennially flowing sites. Within sites, the percentage of marked *N. burrowsius* that were recaptured was generally highest for habitats where the greatest proportion of fish was captured. Other studies have also shown an association between reduced fish movement and high levels of aggregation and have related this to high habitat quality (Essington & Kitchell 1999; Harvey et al. 1999; Crook 2004). Thus, it appears that *N. burrowsius* have high rates of movement, indicated by low recapture rates, unless they occupy habitat of high quality. High levels of movement can be risky as adverse inter-specific encounters may be higher when moving through unfamiliar habitat (Fahrig 2001; Hixon et al. 2002). Thus, the more frequently individuals are required to move due to reduced habitat quality, the greater the potential risk to their survival. Moreover, if suitable habitat is not within dispersible distances, or if dispersal barriers are present, populations are likely to be further adversely effected.

The most important finding arising from my investigations into the distribution of *N. burrowsius* within sites was that the level of fluctuation in abundance within habitats could be related to the presence of deleterious factors at the sites. For instance, at Te Roto Repo o Tawera, observations over the course of this study found few factors likely to threaten population security. The surrounding land was not intensely managed and disturbance to the waterway was low. This was reflected in the stability of distribution patterns at Te Roto Repo o Tawera, with a greater proportion of *N. burrowsius* being consistently caught in a series of pools along a tributary of the main waterway. In contrast, large fluctuations in relative abundance were observed in at least one habitat at the other three sites and were associated

with drought events, stock damage, and the presence of *A. australis*. The mechanism generating these changes is likely to involve movement in response to changes in habitat quality. However, it may also involve mortality, although the total numbers of *N. burrowsius* caught at sites actually increased between April and May 2002, despite similar sampling effort being used. If individual movement proves to be the main determinant of abundance fluctuation patterns within sites, it indicates that *N. burrowsius* is responsive to changes in environmental conditions. Within site changes in distribution may be an early indication that individuals are being adversely affected, which could be useful information in assessing population security.

Conclusion

The identification of objective assessment indictors of conservation status, which require only elementary data, will be essential in on-going conservation efforts for fishes (Dulvy & Renyolds 2002). Increasingly, monitoring is being conducted by volunteer groups, non-scientific agencies and quickly trained personal, using simplified methods (Firehock & West 1995). This should be welcomed as it may be a necessity in large scale monitoring. However, it is important that data collected yield meaningful information, in addition to the techniques being robust and straightforward. My study suggests that estimates of population size and general CPUE trends may be insufficient to adequately determine *N. burrowsius* population security. However, sampling multiple habitats within a site and comparing the proportion of fish caught may provide additional information that can be readily interpreted and related to the presence of potentially deleterious factors. Furthermore, areas of high aggregation within a population's extent, where tag recapture rates are high, may indicate high quality habitat, the recognition of which could focus conservation effort. Thus, overall, the results of this study are interpreted as indicating that Hororata Spring is the least secure of the sites studied, and Te Roto Repo o Tawera the most secure, i.e. this latter site has the greatest likelihood of continued persistence given its current circumstances.

SECTION 2

Population characteristics

At top, a large mudfish caught at St Andrews Drain. At bottom, a small pool in the tributary stream at Te Roto Repo o Tawera where the highest densities of mudfish were caught.

Chapter 5.

Divergent dynamics in *Neochanna burrowsius* populations: forced and constrained size-structures.

INTRODUCTION

The size-structure of a population is a combined result of individual growth, reproductive success, and mortality. Thus, size-structures can illustrate important differences in population dynamics and are a reflection of the prevailing ecological situation and environmental challenges faced by a population. Many intrinsic and extrinsic factors can structure a population, including size-selective predation, life-history traits, and environmental variations (Rodd & Reznick 1997; Holmgren & Appelberg 2000). Growth is especially important, as an individual's size will influence its fecundity, susceptibility to predation, and its physiological response to disturbance (Krause et al. 1998). Fish have indeterminate growth and show considerable plasticity in energy allocation to growth and reproduction (Weatherley & Gill 1987). This allows them to adapt to, and compensate for, potentially limiting factors. Thus, local adaptation can occur in response to strong selective pressure on populations, such as disturbance or predation (Resh et al. 1988; Poff & Ward 1989; Brönmark et al. 1995). Indeed, life-history adaptation has been described as the 'ecological key' that permits fish to persist in adverse environments (Spranza & Stanley 2000).

Despite the diversity of ecological situations in nature, several demographic patterns occur persistently within fish populations. Populations of 'stunted' fish occur throughout the world and this phenomenon has received experimental and theoretical attention, most commonly for species with fisheries value. Stunting is often related to overpopulation that leads to intense intra-specific competition and reduced growth rate (Holopainen & Pitkänen 1985; Brönmark et al. 1995; Holopainen et al. 1997). An interesting example of extremely size-structured populations is observed in Arctic charr (*Salvelinus alpinus*), where populations within the same lake exhibit stable bimodal size-structures, with stunted and giant sized individuals co-occurring, sometimes with little gene flow between the two morphs (Adams & Huntingford 2004). The mechanisms behind these stable and divergent demographic patterns have been

related to life-history traits, food resources, cannibalism, parasite loads, presence of predators and seasonal patterns in disturbance (Rikardsen & Elliott 2000; Claessen et al. 2002).

Most of the literature regarding these issues originates from Northern Hemisphere areas where winter ice cover reduces dissolved oxygen concentrations, resulting in frequent 'winterkills', and the removal of intolerant piscivorous fish species. In these situations, the presence or absence of gape-limited predators is a strong determinant of prey fish size (Brönmark et al. 1995). A well studied example of size dimorphism is the crucian carp (*Carassius carassius*). This species is one of the most hypoxia tolerant vertebrates due to both behavioural and physiological adaptations and is often the only species present in hypoxic ponds throughout Eurasia, being able to survive complete anoxia for periods of five to six months (Holopainen et al. 1997). *C. carassius* are also vulnerable to predation and these hypoxic ponds provide a refuge, resulting in high-density, stunted populations (Holopainen & Pitkänen 1985; Brönmark et al. 1995; Holopainen et al. 1997). Indeed, practically no overlap in size-structure occurs between *C. carassius* populations in the presence or absence of predaceous species (Piironen & Holopainen 1988).

Canterbury mudfish (*Neochanna burrowsius*), the focus of my study is also tolerant of low dissolved oxygen levels and can persist in habitats in which complete anoxia has been recorded (Eldon 1979a). Periods of hypoxia most commonly occur during summer as *N. burrowsius* habitats dry up, although during winter months ice can cover some habitats and in such cases dissolved oxygen is depleted (Eldon 1979a). Further, *N. burrowsius* populations occur in the presence or absence of shortfin eel (*Anguilla australis*), a large opportunistic predator. Thus, this study has many similarities with Northern Hemisphere examples. In this chapter I hypothesised that site attributes (Table 1.2), such as the presence of predators and differences in hydrological regime would be reflected in *N. burrowsius* population characteristics. I demonstrate that populations of *N. burrowsius* occurring in differing ecological situations show divergent population dynamics. However, the situations in which particular dynamics occur in *N. burrowsius* populations differ from Northern Hemisphere examples. Thus, this study sheds new light on the underlying mechanisms structuring fish populations.

METHODS

During the austral autumns of 1999, 2001 and 2002, I undertook field surveys at four sites to determine the population density and size-structure, as well as the individual growth and condition of *N. burrowsius*. Detailed information on the study sites is provided in Chapter 1. Key attributes relevant to this chapter are that predatory *A. australis* were present at Hororata Spring and Dog Kennel Stream, which had seasonally intermittent flow and could dry up during summer. Whereas, Te Roto Repo o Tawera and St Andrews Drain perennially flowed and did not contain predatory fish species. Sampling occurred in autumn, largely to avoid stressful summer conditions and spring spawning periods. However, this timing also provided information on recruitment success, condition, and survival over summer.

Captured fish were anesthetised with approximately 0.5 x 10^{-5} g/L of 2-phenoxyethanol in stream water. Total length (TL) of fish was measured to the nearest half millimetre, and weight to the nearest 0.1 gram using an Ohaus Scout II field balance. Refer to Chapter 2 for further details of fish capture and handling methods. Population structure was compared using length – frequency histograms with 5 mm intervals, following the conventions outlined in Nielsen & Johnson (1983). The density of fish captured, and the density of fish estimated to be present using the Peterson method of mark-recapture (Krebs 1998), were also compared between sites (see Chapter 2).

Fish condition indices are used as a measure of fish 'health' and are considered an integrative measure of environmental conditions (Vila-Gispert & Moreno-Amich 2001). The relationship between length and weight of fish is commonly modelled by the power function and parameters estimated by linear regression based on logarithms (Nielsen & Johnson 1983). A large number of indices have been used to assess fish condition, and selection of a suitable method requires careful consideration of the data set (Bolger & Connolly 1989). In this study, the slope of the length – weight regression did not always fit the ideal assumption of isometric growth (slope = 3.0), which precluded the use of the commonly employed Fulton's Condition Factor (K; Cone 1989). Instead, the condition of each fish was calculated using the relative condition factor (Kn) by the equation $Kn = W/a{\cdot}L^{b}$ (Le Cren 1951; Anderson & Gutreuter 1983), where Kn is relative condition, W is weight (g), L is length (mm), a is the intercept, and b is the slope of the regressed power equation. This factor compensates for allometric growth, i.e. when body shape changes as a fish grows. An advantage of relative condition is that means and standard deviations of Kn provide a better basis for statistical comparison than do tests comparing values for a and b in the length – weight equation. A further practical

advantage of Kn is that average fish of all lengths and species have a value of 1. However, the use of the relative condition factor is only valid for comparisons made within a sample (Cone 1989). Thus, the values of *a* and *b* were calculated from regressions for all individuals captured at all sites in this study to enable between year and site comparisons.

Unfortunately, attempts to estimate growth rates of individually marked fish using visual implant tags was hampered by poor tag recaptures, resulting in insufficient data to satisfactorily compare growth rates between intermittent and perennial sites. Thus, sagittal otoliths were extracted from *N. burrowsius*, retained after accidental death, from Te Roto Repo o Tawera, Hororata Spring and Dog Kennel Stream during the period 1999 – 2002. Dead fish were decapitated and their heads digested for 24 hours at 37 °C in a solution of Pancreatin (2.2 g/l) dissolved with 17.5 g/l KOH in distilled water. After enzyme digestion of surrounding tissue and structures, otoliths were washed with distilled water and cleared with methyl salicylate. Otolith rings were examined under x35 magnification but were not found to be a reliable measure of age because they exhibited irregular and apparently non-seasonal changes in density. Eldon (1979c) also concluded that *N. burrowsius* could not be aged from otoliths as growth rate after the first year was too slow, rendering interpretation of apparent annual rings meaningless.

Otolith growth is less variable than fish growth (Campana & Casselman 1993). This is because the rate of calcium absorption into the crystal lattice of the otolith is constant (Pannella 1974). Studies conducted on guppies (*Poecilia reticulata*; Reznick et al. 1989) and striped bass (*Morone saxatilis*; Secor & Dean 1989) have demonstrated that slowly growing fish have larger otoliths than equally-sized but faster growing fish. Thus, otolith weight is directly related to age and can be used as a reliable measure of relative growth (Reznick et al. 1989; Fletcher 1991). Otolith weight was measured to the nearest 0.001 mg with a Mettler Toledo microbalance.

Statistical analysis of the relationship between fish length and otolith weight was conducted in Prism 3.0 (GraphPad Software 1999) using linear regression on natural log transformed data. The slope and intercept of this relationship was compared using homogeneity of slopes test and ANCOVA to examine differences in growth rate between populations.

RESULTS

Population density

The density of *N. burrowsius* (Table 5.1) was higher in perennial sites and lower in intermittent sites (ANOVA: $F_{(3, 15)} = 14.0$, $p < 0.001$). St Andrews Drain had the highest density, followed by Te Roto Repo o Tawera, whereas, the density at Hororata Spring and Dog Kennel Stream were very low. Similar patterns occurred in the density of fish caught and the density estimated from mark recapture data (Table 5.1).

Table 5.1. Average densities of *N. burrowsius* in habitats within the four sites, Te Roto Repo o Tawera, Hororata Spring, St Andrews Drain, and Dog Kennel Stream. Estimated densities were obtained using the Peterson method on mark recapture data (see Chapter 4). Capture density is the number actually caught. The standard error on means is given in brackets.

N. burrowsius	Site			
	Tawera	Hororata	St Andrews	Dog Kennel
Estimated density (no./m^2)	4.7 (0.73)	1.4 (1.07)	4.5 (0.88)	3.2 (1.25)
Capture density (no./m^2)	0.42 (0.088)	0.07 (0.038)	0.80 (0.251)	0.27 (0.066)

Population structure

The size-structure of *N. burrowsius* populations varied according to flow permanence and presence or absence of *A. australis* (Figure 5.1). At Te Roto Repo o Tawera and St Andrews Drain, the perennial sites without *A. australis*, there was a substantial proportion of large *N. burrowsius*. In comparison, few *N. burrowsius* greater than 100 mm TL were found at Hororata Spring and Dog Kennel Stream, although large numbers of small fish were present at these intermittent sites. These widely divergent patterns separated the perennial from the intermittent sites. Thus, dense populations of large fish, with apparently poor recruitment, occurred in habitats with continuous flow, whereas in seasonally hypoxic habitats, the opposite population characteristics were found.

A juvenile cohort was present at all sites, yet was most apparent at Hororata Spring and Dog Kennel Stream. Length frequency histograms with 2 mm size class intervals were used to identify juveniles (< 65 mm TL) at each site. The juvenile cohort modes were 56, 58, 46, and 50 mm TL for Te Roto Repo o Tawera, St Andrew Drain, Hororata Spring, and Dog Kennel Stream, respectively. Differences in population structures between flow regimes were also relatively stable between years (Figure 5.2). At intermittent sites at least 75 % of fish were < 85 mm TL in all years, whereas at least 75 % of fish in perennial sites were > 70 mm TL in all years. However, the size composition of *N. burrowsius* populations at intermittent sites fluctuated more than in perennial sites (Figure 5.2), indicated by the 25 – 75 % quartile, which did not consistently overlap during the three sampling periods in intermittent sites, yet did so in perennial sites.

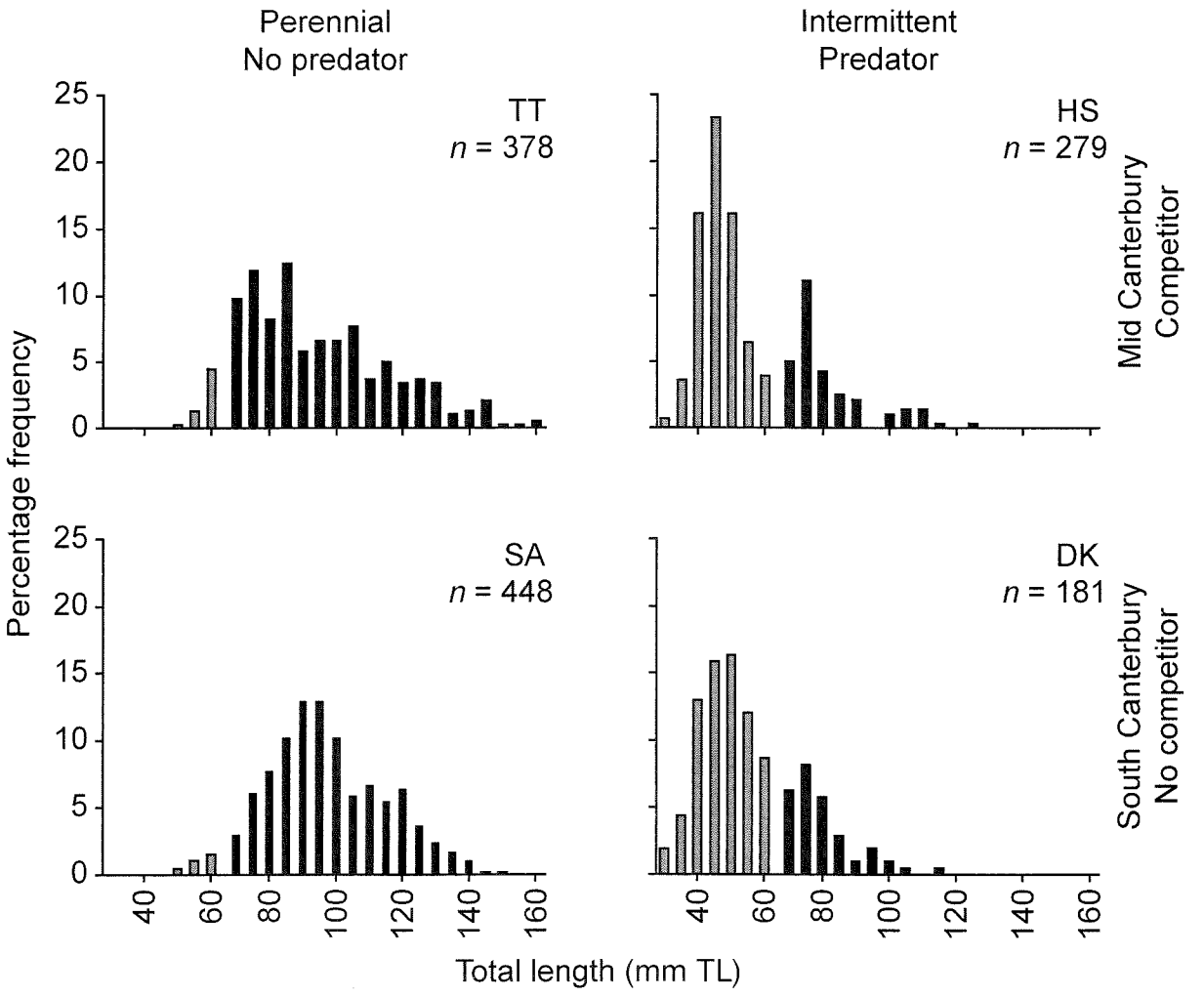

Figure 5.1. *N. burrowsius* population structure at two intermittent (HS = Hororata Spring; DK = Dog Kennel Stream), and two perennial sites (TT = Te Roto Repo o Tawera; SA = St Andrews Drain), pooled over three years and shown as percentage frequency of each 5 mm size class, with grey bars indicating juvenile fish < 65 mm TL.

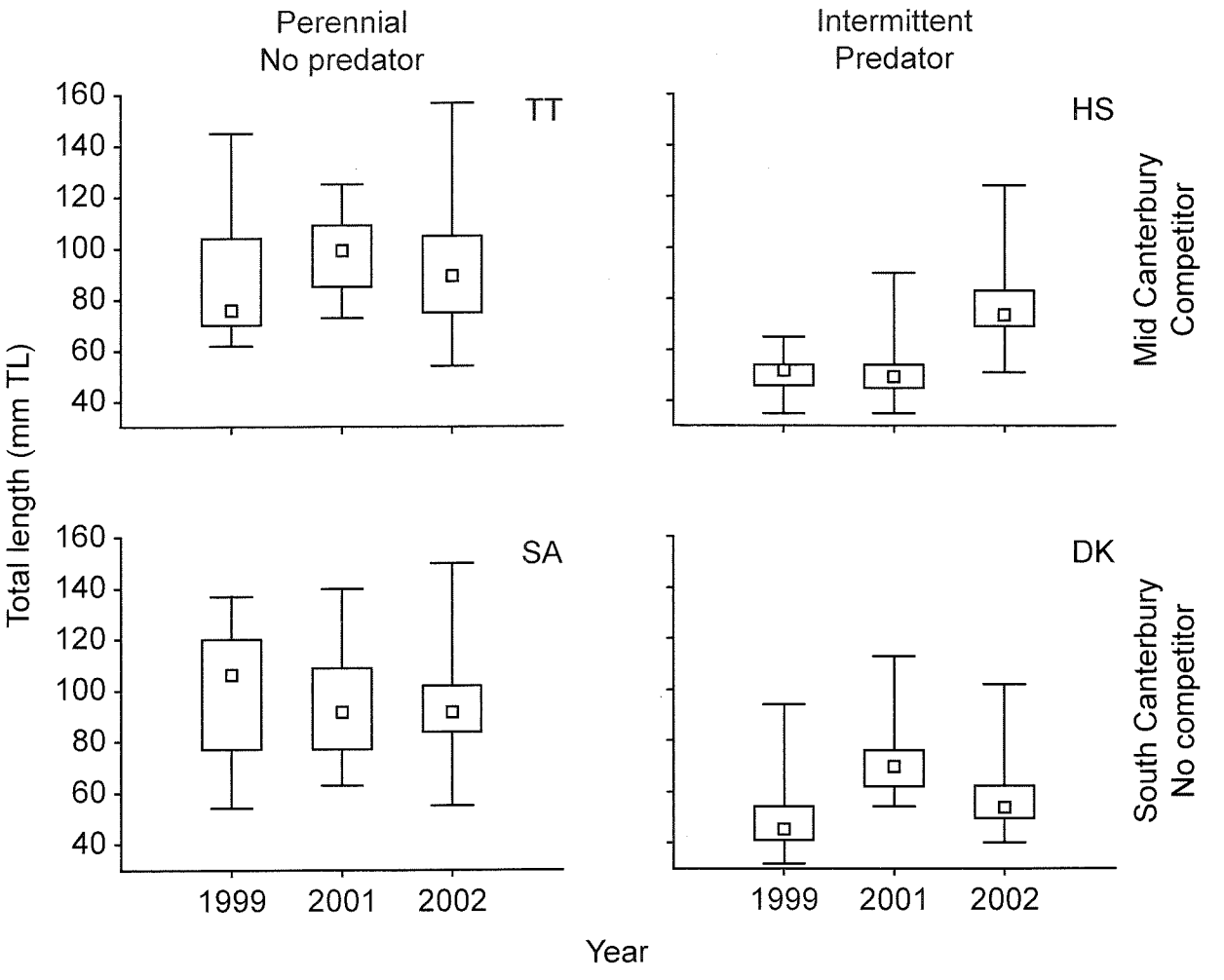

Figure 5.2. Length distributions (mm TL) of *N. burrowsius* for each site for each sample period at intermittent (HS = Hororata Spring; DK = Dog Kennel Stream), and perennial sites (TT = Te Roto Repo o Tawera; SA = St Andrews Drain). Box plots indicate median (centre point), 25 and 75 % quartiles (box) and the minimum and maximum lengths (whiskers).

Within sites, *N. burrowsius* individuals downstream of core adult habitat (see also Chapter 4) were often shorter, especially in dense populations. This was obvious from surveys at St Andrews Drain (Figure 5.3; ANOVA: $F_{(3, 382)} = 15.55$, $p < 0.001$), and was also observed during spot surveys at Te Roto Repo o Tawera.

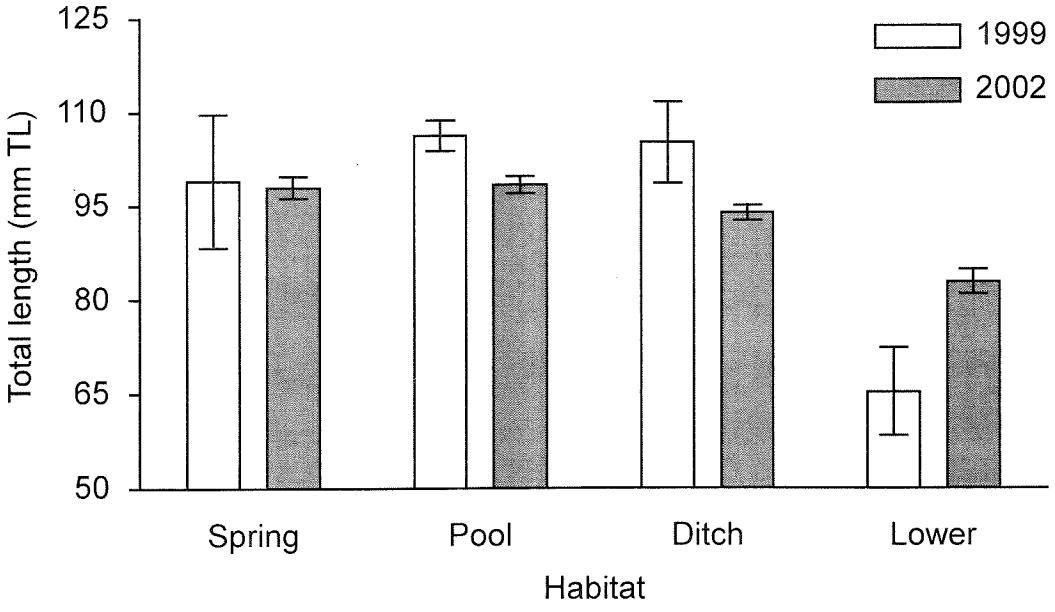

Figure 5.3. Longitudinal patterns in *N. burrowsius* length at St Andrews Drain in 1999 and 2002. Habitats are plotted upstream to downstream; the waterway originating at the Spring.

Relative condition

Sites that experienced the greatest hydrological fluctuations (intermittent flow) also appeared to have the greatest annual variation in condition. The coefficient of variation in relative condition (Kn) in perennial sites was 0.08 for both Te Roto Repo o Tawera and St Andrews Drain; compared with 0.20 and 0.22 at the intermittent Hororata Spring and Dog Kennel Stream sites respectively.

Regional synchronicity in condition fluctuations was observed with Mid and South Canterbury sites showing opposing temporal peaks. For example, in 1999 Te Roto Repo o Tawera and Hororata Spring had high condition, relative to other years, with fish having higher condition than southern sites in that year (Figure 5.4). The opposite pattern was seen in 2001, with *N. burrowsius* in South Canterbury having consistently higher condition.

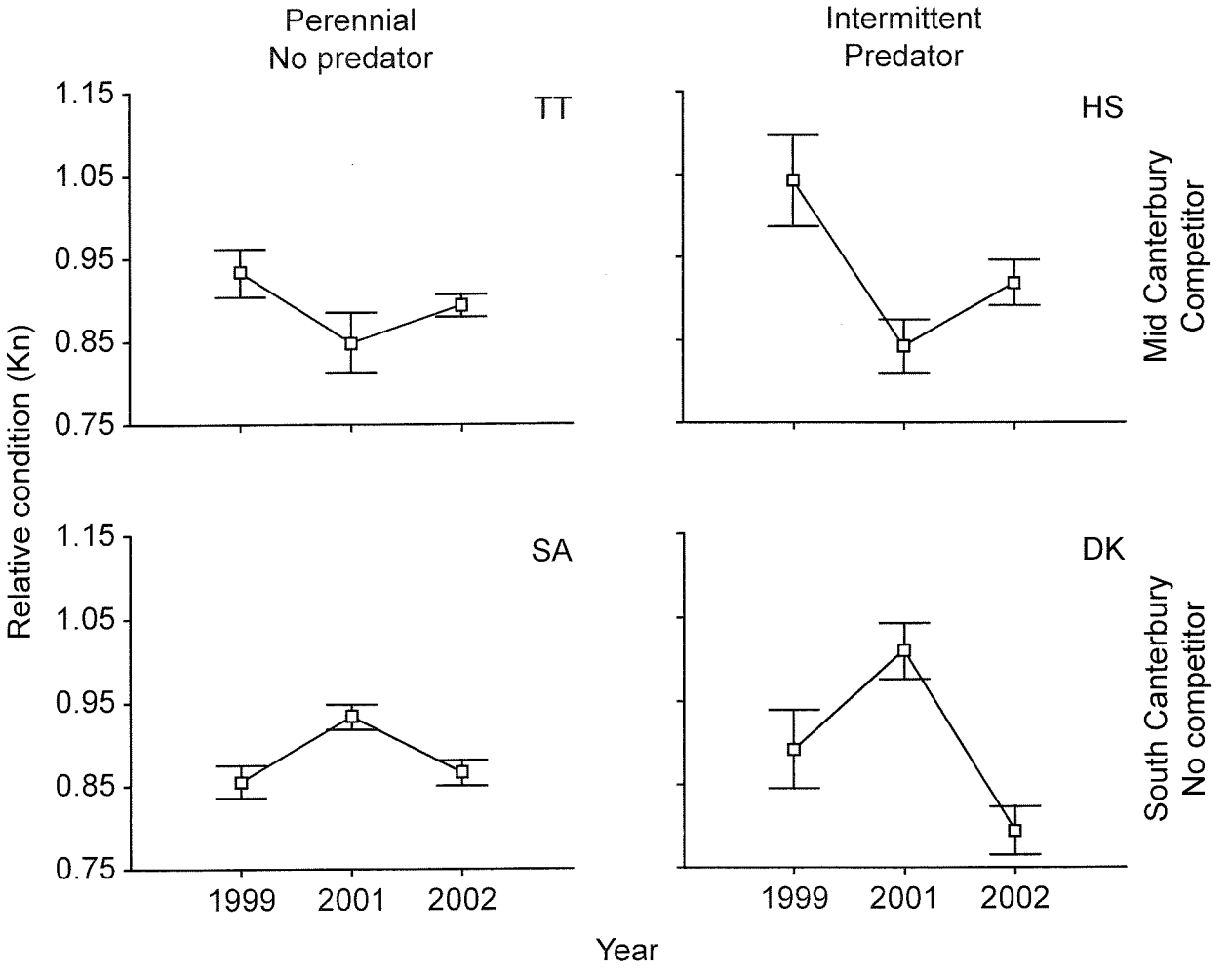

Figure 5.4. Relative condition (mean Kn ± 1 SE) for intermittent (HS = Hororata Spring; DK = Dog Kennel Stream), and perennial sites (TT = Te Roto Repo o Tawera; SA = St Andrews Drain), located in Mid and South Canterbury, during autumn 1999, 2001, and 2002.

Otolith characteristics

The slope of the relationship between otolith weight and *N. burrowsius* length was significantly different amongst the three populations tested (homogeneity of slopes test: $F_{(2, 20)} = 5.8$, $p = 0.01$). The greatest slope was seen at Hororata Spring, and Dog Kennel Stream was similar, indicating that *N. burrowsius* in intermittent sites had heavier otoliths at a shorter fish length than *N. burrowsius* at the perennial Te Roto Repo o Tawera site (Figure 5.5). This indicates that *N. burrowsius* from intermittent sites were older at any given length and thus had grown more slowly than *N. burrowsius* from Te Roto Repo o Tawera. These differences could be even larger as oxygen deficiency, which occurs in intermittent sites, can induce calcium resorption in otoliths (Mugiya & Uchimura 1989).

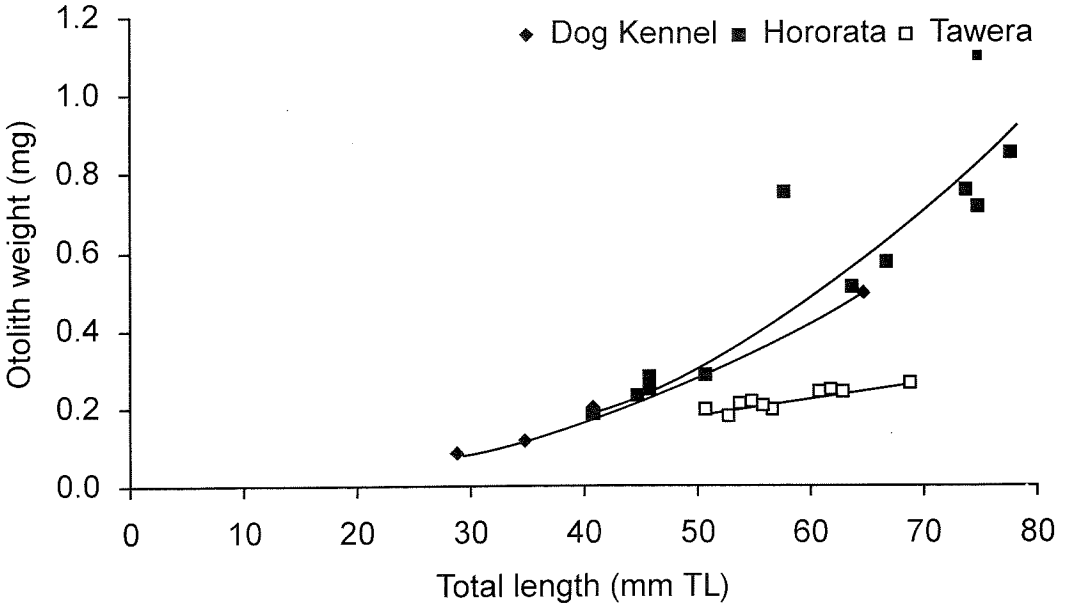

Figure 5.5. Relationship between *N. burrowsius* total fish length and otolith weight fitted by power equations. Closed and open symbols represent otolith data from populations experiencing intermittent and perennial flows, respectively. Fish were collected during the period 1999 – 2002.

DISSCUSION

In this study, I found that intermittently flowing sites with *A. australis* present contained low-density populations of *N. burrowsius*, with individuals of small size, having fluctuating and often high condition, high apparent recruitment, yet low growth rate. This contrasts with populations in perennial sites in the absence of *A. australis* where individuals were large, but had lower average condition and recruitment, yet had high growth rates and density. These distinct population dynamics were stable over the period of this study. Additionally, they appear stable over longer time periods, as differences in maximum adult size were also apparent in the 1970s. Sampling conducted at Hororata Spring in February 1975 found that maximum adult size was 114 mm, whereas at Te Roto Repo o Tawera individuals with lengths of 146 mm and 155 mm were caught in February 1976 and 1978, respectively (G. A. Eldon unpublished data). Moreover, analysis of otolith area and fish length also indicates that growth rates of *N. burrowsius* were lower at Hororata Spring, than at Te Roto Repo o Tawera in the 1970s (G.A. Eldon unpublished data).

Similar dichotomies in population structure have been found in several well studied freshwater fish species including, *S. alpinus* (Johnson 1994; Power et al. 2000), *C. carassius*

(Holopainen & Pitkänen 1985; Brönmark et al. 1995; Holopainen et al. 1997), and pumpkinseeds (*Lepomis gibbosus*; Fox & Keast 1990, 1991). Such population structures have been generated through manipulation, and return after perturbations, indicating that stable population dynamics underlie these patterns (Tonn & Paszkowski 1986; Piironen & Holopainen 1988; Brönmark & Miner 1992; Johnson 1994; Holopainen et al. 1997). Furthermore, differences in the maximum size attained are due to environmental factors rather than genetic differentiation (Heath & Roff 1987).

Multiple descriptions for these divergent size-structures occur in the literature, including stunted or normal, dwarf or giant, and size dimorphic (Johnson 1994; Brönmark et al. 1995; Holopainen et al. 1997; Parker et al. 2001; Claessen et al. 2002). To aid comparison, in this chapter I describe the size-structure pattern as found in Hororata Spring and Dog Kennel Stream as 'constrained', and that of Te Roto Repo o Tawera and St Andrews as 'forced' (Figure 5.6).

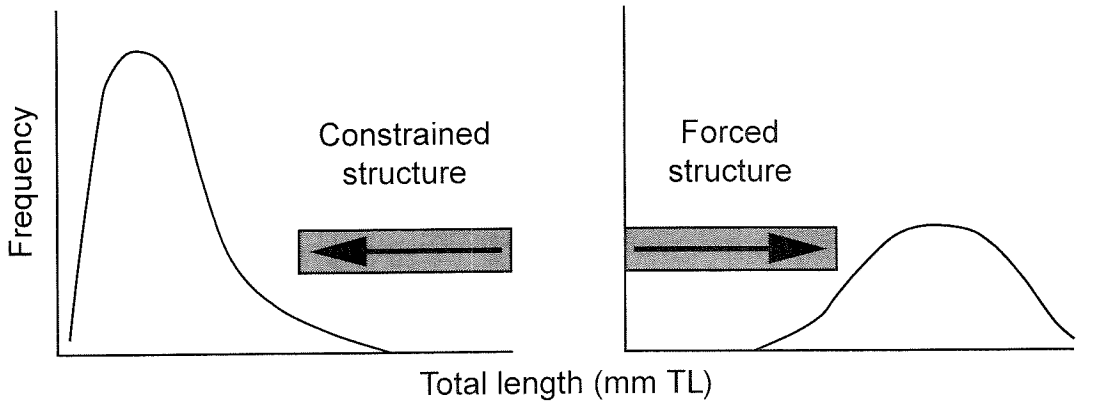

Figure 5.6. Summary of divergent population structures and the proposed classifications of 'constrained' and 'forced' structures. Grey boxes indicate size classes suppressed or absent in populations. Arrows indicate direction of size selection pressure.

Mechanisms generating a forced structure

The suppression of juveniles by larger conspecifics purportedly explained a forced population structure in *S. alpinus* (Johnson 1994). Similarly, at Te Roto Repo o Tawera and St Andrews Drain, high adult *N. burrowsius* density may result in displacement of juveniles to peripheral habitats. The presence of smaller *N. burrowsius* in 'unsuitable' downstream areas of St Andrews Drain and in waterways surrounding Te Roto Repo o Tawera supports this explanation. However, considering the recruitment potential of such large adult populations, this mechanism cannot fully explain the near absence of juvenile fish in perennial habitats.

There is strong evidence that gape-limited predation produces forced population structures in prey species (Brönmark et al. 1995; Rodd & Reznick 1997). The presence of a gape-limited predator can even lead to an increase in prey fish size due to an induced morphological response, as observed in *C. carassius* (Brönmark et al. 1995). This results from increased resource allocation to growth in size and body depth, leading to an effective predator defence mechanism by allowing avoidance of the 'predator window' (Brönmark & Miner 1992; Brönmark et al. 1995; Pettersson & Brönmark 1997; Claessen et al. 2002). Cannibalism has also been suggested as a mechanism capable of maintaining size dimorphism in population structures (Claessen et al. 2002). Cannibalistic fish are invariably gape-limited, suggesting a common mechanism in structuring forced populations. Cannibalism is common in fish (Juanes 2003) and occurs in galaxiids, as they are opportunistic predators, and could be a form of recruitment suppression (Bonnet & Lambert 2002; Allibone 2003). *N. burrowsius* may consume large quantities of eggs in captivity (Chapter 10), and *N. burrowsius* eggs have been found in the stomachs of wild *N. burrowsius* (Cadwallader 1975a; Eldon 1979b). Furthermore, brown mudfish (*Neochanna apoda*) cannibalise eggs and fry in the wild (Eldon 1978). It is likely that cannibalism is dependent on adult density and habitat structure (Chapter 10). Thus, gape-limited predation, whether intra- or inter-specific, is likely to be the ultimate structuring mechanism in forced populations, via the removal of small individuals, either directly through consumption or indirectly via juvenile movement to avoid predation, or by inducing increased allocation to growth.

Mechanisms generating a constrained structure

The key point of difference between my findings and that of others is that constrained size-structures occurred in populations of low-density and in the presence of predators. Stunting is most often observed in prey populations that exist in the absence of piscivorous fish, often due to seasonal occurrence of low dissolved oxygen causing 'winterkill' of predators (Tonn & Paszkowski 1986; Brönmark et al. 1995). Low growth rates are widely considered the ultimate mechanism producing constrained size-structures, and to a lesser degree reduced overall life expectancy (Ylikarjula et al. 1999; Parker et al. 2001). However, several mechanisms may lead to this phenomenon in fish populations (Fox & Keast 1990, 1991; Jansen & Mackay 1991; Holopainen et al. 1997; Margenau et al. 1998).

Due to the occurrence of constrained population structures in high-density populations, a prevailing suggestion is that intra-specific competition is the proximate mechanism generating

these patterns (Piironen & Holopainen 1988; Holopainen & Pitkänen 1985; Holopainen et al. 1997). However, there is still debate on the role of intra-specific competition in directly generating constrained population structures because manipulations to decrease densities have not always improved growth or fish size (Margenau et al. 1998). Further, fish restocked into a pond that previously containing a constrained population developed a similar size structure after two years (Piironen & Holopainen 1988). Conversely, translocating stunted fish into habitats with naturally less dense populations does result in increased growth and size (Holopainen et al. 1997; Margenau et al. 1998). These manipulations suggest the importance of abiotic environmental factors. Furthermore, there is uncertainty as to how intra-specific competition can specifically lead to high mortality of larger fish. It is suggested that high intra-specific competition for limited resources could result in the low survival of older fish due to a relatively poorer nutrition situation (Holopainen et al. 1997; Piironen & Holopainen 1988). However, larger fish, due to lower metabolic rate per unit of biomass can be expected to survive periods of fasting in better condition than smaller con-specifics, as has been demonstrated in over-wintering bluegills (*Lepomis macrochirus*; Cargnelli & Gross 1997) and in black mudfish (*Neochanna diversus*; McPhail 1999). Moreover, intra-specific competition tends to favour larger bodied individuals (Holopainen et al. 1997; Post et al. 1999). Thus, further work is required before accepting intra-specific competition as the sole mechanism structuring constrained populations.

Unlike intra-specific competition, seasonal hypoxia imposes well-defined size-dependent physiological constraints on maximum size (Persson et al. 2000). Small fish have lower overall oxygen requirements and high surface area to volume ratios, thus have a greater tolerance to hypoxia. Thus, it is generally agreed that smaller, slower-growing individuals are better able to tolerate the environmental stress associated with hypoxia (Casselman & Harvey 1975; Chapman et al. 1996; Margenau et al. 1998). In addition to placing a physiological constraint on maximum size, extreme periods of hypoxia can cause size-dependent mortality. Fox & Keast (1990) concluded that a constrained size-structure in *L. gibbosus* was due to high annual mortality of larger fish, not stunted growth. Further, Ciepielewski (1967), cited in Holopainen & Pitkänen (1985), suggested that a constrained size-structure in *C. carassius* was due to frequent winterkills selectively affecting older age groups, rather than intra-specific competition. In cases of winter hypoxia, differences in the intensity and duration of hypoxic events were related to lake size and depth (Piironen & Holopainen 1988; Johnson 1994). In this respect, constrained size-structures of *S. alpinus* in Greenland were only found in lakes < 8 m deep (Riget et al. 2000). Superficially, this situation differs from that in which *N.*

burrowsius is found, however, it is likely that the physiological constraints imposed by hypoxia are similar, irrespective of whether such events occur in winter or summer.

Despite this evidence, and although seasonal hypoxia frequently occurs in many habitats containing constrained populations throughout the world, it is not universally accepted as a mechanism structuring populations (Holopainen & Pitkänen 1985). Nevertheless, it is likely that physiological constraints imposed by hypoxia are the underlying mechanism structuring many of these populations. It is often difficult to untangle the effect of resource limitation, from that of seasonal hypoxia directly, as survival during such periods of stress can require additional energy resources (Finstad et al. 2002). The requirement to store glycogen, instead of allocating it to growth or reproduction, has also been proposed as a structuring force resulting in constrained populations (Brönmark et al. 1995). For example, *C. carassius* must accumulate reserves to allow winter fasting and the use of alternative, but less efficient metabolic pathways during periods of anoxia (Brönmark & Miner 1992; Holopainen et al. 1997). Fish are relatively plastic in their growth patterns and can compensate for periods of reduced resources (Weatherley & Gill 1987; Holopainen et al. 1997). Additionally, phenotypic plasticity in patterns of energy allocation have been well documented and are related to resource fluctuations (Jansen & MacKay 1991). In comparisons between dichotomous population structures, constrained *L. gibbosus* and yellow perch (*Perca flavescens*) populations experiencing winter hypoxia allocate proportionally more energy to gonadal development and may mature earlier than populations in normoxic conditions (Fox & Keast 1991; Jansen & Mackay 1991; Bertschy & Fox 1999). Thus, the low growth rate of constrained populations of *N. burrowsius* in my study may be due to plasticity in resource allocation required to overcome hypoxia, rather than overall resource limitation. This is indicated because although individuals occurring in constrained populations had low growth rates, they also had high relative condition. This suggests *N. burrowsius* were maintaining short-term energy reserves in preference to growing in length, which is a largely irreversible allocation of reserves. Fat levels and condition of *N. burrowsius* decrease during autumn and remain low through winter, till spawning in late winter or early spring (Eldon 1979c). Therefore, condition levels during autumn are likely to indicate the level of energy reserves potentially available for gonad development over winter. Thus, the occurrence of high relative condition is likely to indicate a greater allocation to reproduction. Furthermore, *N. burrowsius* in constrained populations do appear to become sexually mature within their first year, despite a very small size. During September 2000, five male *N. burrowsius* with flowing milt were caught at Hororata Spring. The sizes of these fish ranged from 50 – 62 mm

TL and on average were 55 mm TL. This is less than the length usually quoted as indicating an adult *Neochanna* (i.e. > 65 mm; Ling et al. unpublished). Thus, stunting does not affect the age at sexual maturity, although it is likely to affect potential fecundity.

A further result of my study was that despite greater fluctuations in relative condition of *N. burrowsius* from constrained populations, population structures were relatively stable. Thus, despite having high condition in some years, substantial growth in length did not occur. This is surprising as increased length has fitness benefits via substantially increased potential fecundity (Eldon 1979c). One possible explanation is that although the severity of seasonal disturbance may change from year to year, any response may be dampened due to *a priori* allocations of energy. Furthermore, local adaptations to hypoxic environments can be passed on to offspring (Chapman et al. 2002b), suggesting that energy allocation patterns may become fixed within a population. Thus, local adaptation and/or plasticity in resource allocation may allow *N. burrowsius* to persist in the face of seasonal but unpredictably severe disturbance.

Conclusion

N. burrowsius populations exhibited divergent population structures and dynamics similar to those observed in many other fish species worldwide. However, differences in the circumstances of my study may provide insight into the ultimate mechanisms generating these divergent patterns. Most importantly, constrained populations of *N. burrowsius* had low densities, thus intra-specific competition was unlikely to be a relevant mechanism. I suggest that dichotomous size-structures, such as observed in *N. burrowsius*, can occur independently of a population's density, whereby density may act only to strengthen or weaken characteristic population structures. Overall, this study supports others by indicating that forced population structures result from gape-limited predation, in the case of *N. burrowsius* via cannibalism. However, a constrained population structure in *N. burrowsius* is not, as suggested in other studies, a result of intra-specific competition, but rather is likely due to physiologically based constraints applied by seasonal hypoxia. It is possible that a closer examination of Northern Hemisphere examples of constrained size-structures will show that the size selectivity of winterkills plays a greater role in structuring populations than is currently recognised.

Chapter 6.

Disease, parasitic infection, and deformity in *Neochanna burrowsius* populations.

INTRODUCTION

Fish regularly encounter pathogens in their habitat and generally have adequate resistance unless weakened by other agents or wounded (van Duijn 1973). Thus, disease in fish usually involves several contributing factors acting to weaken an individual, such as, malnutrition, stress, parasites, bacteria, viruses, as well as, genetic attributes (Hoffman 1973). Furthermore, disturbed environments often place frequent physiological stress on the fish inhabiting them. Such stress can cause disruption to homeostasis, increased cortisol levels, and require the diversion of energy into maintaining homeostasis, leading to decreased resistance to disease (Van Weerd & Komen 1998). Disease in wild fish populations has been associated with stress induced by temperature changes, low dissolved oxygen, eutrophication, sewage and synthetic pollution (Snieszko 1974).

Additionally, resource and environmental stress may act to exacerbate underlying genetic susceptibilities to deformity. Abnormal morphology within teleosts occurs frequently and can have genetic, environmental, nutritional, and/or pathogenic causes (van Duijn 1973; Koumoundouros et al. 2001; Beraldo et al. 2003). In general, Canterbury mudfish (*Neochanna burrowsius*), the focus of this study, has very low genetic heterozygosity (Davey et al. 2003), which may be reflected in higher rates of malformation. Eldon (et al. 1978) commented that fin malformation in *N. burrowsius* was common enough to hinder fin clipping during a mark-recapture study. Although the ultimate causes of deformity are debated, particular characteristics such as, whether abnormalities occur symmetrically, have been used to differentiate between inherited and environmentally induced deformities (Beraldo et al. 2003). Common environmental limiters, e.g. high water temperature and low dissolved oxygen levels, can induce deformities of the spine and head regions (van Duijn 1973), and have been associated with deformity in early life stages of *N. burrowsius* during laboratory hatching experiments (Eldon 1979c). Low dissolved oxygen levels are a frequent characteristic of *N. burrowsius* habitat (Eldon 1979a). Thus, it is possible that *N. burrowsius*

experiences a high rate of deformity due to the combined influence of low genetic diversity and poor environmental conditions during early development. Furthermore, the lowland habitats that *N. burrowsius* occupies are surrounded by intense agricultural activity. Many of these remnant waterway habitats are an integral part of farming systems, being used for irrigation, drainage, and stock water. Direct stock access to waterways may increase bacterial loadings and reduce water quality. This is likely to be detrimental to *N. burrowsius* as it has been described as a 'clean water species', being sensitive to long term poor water quality (Eldon 1993; Young 1996). Thus, it is hypothesised that the level of agricultural activity surrounding a habitat will be reflected in the health of a *N. burrowsius* population.

Irrespective of anthropogenic effects, the macrophyte dominated, slow flowing waterways that *N. burrowsius* favour can support large numbers of pathogens (McDowall 1990). These habitats also contain numerous invertebrates that may act as intermediate hosts to fish parasites. *N. burrowsius* may thus acquire infection through the consumption of prey, such as, Diptera, micro-crustaceans, and molluscs (McDowall 1990); which can form a large portion of *N. burrowsius* diet (Eldon 1979b). Parasites, by definition, have a detrimental effect on their hosts (Barber et al. 2000). Infection by parasites is a significant source of mortality in fish populations, potentially structuring communities (Holt & Pickering 1985). However, parasites can have wide ranging non-lethal influences on fish behaviour, affecting competitive ability, and social interactions between fish (Barber & Ruxton 1998; Barber et al. 2000). However, the most serious threat is that of increased predation risk. Parasitised fish may be induced to forage more, to acquire additional energy requirements, or they may be debilitated by parasites, rendering them easier targets (Poulin 1993).

Infection by ecto- or endo-parasites has differing implications for host survival (Barber & Ruxton 1998). Endo-parasites are highly dependent on the survival of the host, whereas ecto-parasites do not, and thus, are more likely to cause mortality. Endo-parasites have not been recorded in *N. burrowsius*, possibly due to the small numbers of fish examined; however it is likely that they are parasitised (Blair 1984; Meredith 1985). In the more widespread brown mudfish (*Neochanna apoda*), infection rates from the endo-parasitic nematode *Hedruris spinigera* reached 63 % in a population feeding extensively on the intermediate amphipod host *Paracalliope fluviatilis* (Eldon 1978). In this study I only conducted non-destructive external examinations, thus avoiding intentional mortality, given the conservation status of *N. burrowsius*. Although this method may underestimate parasitic loadings, the easily observed

ecto-parasites, such as the ubiquitous and potentially lethal *Ichthyophthirius multifiliis* are likely to pose the most substantial threat of mortality to their hosts (McDowall 1990).

In this chapter, I investigated the relationship between the apparent health of *N. burrowsius* populations, in terms of incidence of deformity and external infection by pathogens and parasites, and their genetic diversity, as well as the environmental stresses placed on them by water quality and fluctuating hydrological regimes. Four *N. burrowsius* populations were examined, which represented pairs with differing genetic diversity, from habitats with differing levels of agricultural activity and hydrological disturbance. Comparisons between these populations may thus provide an insight into which factors are involved in determining the general health of *N. burrowsius* individuals. I hypothesised that populations with low genetic diversity would exhibit high levels of deformity and that populations experiencing environmental stresses, particularly from anthropogenic sources, would have higher rates of pathogenic infection.

METHODS

N. burrowsius were captured using Gee minnow traps and an electrofishing machine before careful examination by eye for abnormalities at four sites during May 2002. See Chapter 2 for additional details on fish capture and handling. Incidences of externally visible parasites, infection, deformity and other abnormalities were recorded. All *N. burrowsius* were examined for deformity, however, fish previously captured, indicated by the presence of a paint batch, or visual implant tag were not examined for infection. These fish were excluded because the prior handling procedure in April 2002 (Chapter 4) involved treating fish with an anti-pathogenic solution (Chapter 2), which may have influenced infection rates during the intervening period.

Study sites

The four sites used in this study, Te Roto Repo o Tawera, Hororata Spring, St Andrews Drain, and Dog Kennel Stream, have been described as the most important habitat remnants for *N. burrowsius* (Eldon 1993), being fully described in Chapter 1. Genetic studies have found that *N. burrowsius* populations at two of these sites exhibit no detectable genetic diversity (Davey et al. 2003). Site characteristics relevant to this chapter involve the level of agricultural activity and the extent of hydrological disturbance (Table 1.2). In summary, Hororata Spring and St Andrews Drain experienced the most intense agricultural disturbance, with heavy

grazing by sheep and cattle, which had direct access to the water. At these sites stock faeces were commonly seen in the waterway. Furthermore, at Hororata Spring a large, deep pool was used for duck shooting. Scattered grain was used to attract large numbers of ducks to the area prior and during the autumn shooting season. Large numbers of waterfowl also congregated in the ponds during dry summers when these ponds appeared to be the only remaining surface water in the surrounding area. During these periods habitat quality appeared low (Figure 6.1). Large hydrological fluctuations occurred at Hororata Spring and Dog Kennel Stream, whereby habitats frequently dried up in summer and autumn, likely imparting environmentally induced stress. In contrast, Te Roto Repo o Tawera and St Andrews Drain had perennial flow and habitats did not dry up seasonally.

Figure 6.1. A decoy duck floats beside heavily grazed riparian vegetation and a surface scum of manure and algae during late summer when duck and stock numbers were high at Hororata Spring.

External examination

Individual fish health was evaluated by the occurrence of external parasites, infection and injury. Previous observations of these *N. burrowsius* populations guided the development of categories for ailments and disability. Four categories covered apparent bacterial infections, three for parasite types, and another for fungal infections. These categories were grouped for statistical analysis into infection by parasite, bacteria and/or fungi. Further categories described tumours, deformity, injury, abnormal pigmentation, and fish with low condition from unidentifiable causes.

Categories used in the evaluation of bacterial infection were based on readily identifiable symptoms; fin rot (caused by *Bacterioses pinnarum*), 'wire disease' (distinctive, yet unidentified disease involving dark spots and necrosis) and the presence of red spots and blotches (ecchymoses; van Duijn 1973). Fungi were identified by distinguishing hyphae structures. Gross assessment of ecto-parasites present was conducted using categories based on the colour of parasitic cysts (trophont); white, red, and black cysts present were typically approximately 1 mm in diameter. White cysts were identified as *I. multifiliis* with the intensity of infection (number of individual parasites harboured per individual), and their prevalence (the proportion of hosts harbouring at least one individual parasite) being recorded. Other species of parasites observed on or through the skin could have been shallowly placed metacercarial cysts of the fluke *Telogaster opisthorchis* (Poulin 1993), trematodes, or nematodes, such as, *Eustrongylides ignotus* (McDowall 1990).

Water chemistry

Evaluation of water quality was conducted one month before (April), and during (May) the external examination of *N. burrowsius* in 2002. Dissolved oxygen, pH, turbidity and conductivity were measured in the field. Conductivity and pH were measured by an Oakton pH/CON 10 meter and dissolved oxygen using an YSI Model 95 probe. Percentage air saturation of dissolved oxygen was used in statistical analysis as a suitable measure to compare measurements taken at different temperatures (Burleson et al. 2001). Nutrient status was also measured at each site, above and below study areas, one month prior to, and at the time of *N. burrowsius* examination. One litre samples of water were collected, filtered through grade 1 quantitative filter paper and chilled to 4 °C. Measurement of nitrate, nitrite and phosphate were performed with a HACH DR/2000 spectrophotometer using standard photometric methods (HACH 1996). Nitrites were removed, prior to measurement of nitrates,

with bromine water (30 g/l), and 1 % phenol solution. Dilution was used on a freshly prepared sample if concentrations exceeded the range of the meter.

Statistical analysis

The prevalence of disease and parasites were tested by Chi-square analysis to determine if prevalence was independent of site. Correspondence analysis was used to evaluate associations between environmental factors and the physical health of *N. burrowsius*. In addition to the inclusion of mean values for each water chemistry measure (Table 6.3), two rank variables were used to include the influence of grazing and water velocity in the analysis. All statistical analyses were performed using Statistica 6.0 (Statsoft Inc. 2001).

RESULTS

N. burrowsius individuals were infected by parasites, bacteria, and fungi at all sites. The proportion of healthy *N. burrowsius*, with no visible infection, injury, or parasites was highest in Dog Kennel Stream, and lowest in Hororata Spring (Figure 6.2). Overall the prevalence of infection was not independent of population (χ^2 = 238.5, df = 19, $p < 0.001$), indicating significant differences between sites. The prevalence of fungal infection was generally low, with all occurrences appearing to be *Saprolegnia*. This fungus is not an obligate parasite and is only able to infect via existing wounds, infection and/or reduced immunity (van Duijn 1973). *Saprolegnia* was found to be more prevalent in the perennially flowing sites of Te Roto Repo o Tawera and St Andrews Drain (Figure 6.2). The prevalence of bacterial infection was greater than that of fungal infection, being greatest at Hororata Spring (Figure 6.2). During examination any sign of inflammation, ecchymoses and fin erosion was included in the prevalence data, and no attempt to estimate the severity of infection was made. Thus, most individuals with signs of bacterial infection appeared otherwise healthy, and it is likely that their immune responses would have been sufficient to combat the spread of infection. Bacterial infections mainly occurred on the anal and caudal fins, most likely exacerbated by contact with the substratum, and injury through movement (Table 6.1). Of those *N. burrowsius* found to have poor condition, five individuals from St Andrews Drain also displayed similar symptoms likely to indicate the presence of disease. Typically, their skin had a milky turbidity, especially around pores, and the tail region.

Parasites observed on the skin of *N. burrowsius* were predominately the ciliate protozoan *I. multifiliis* (109 cases). This parasite was present in all populations; however it reached a

potentially epidemic level at Hororata Spring where 50 % of *N. burrowsius* captured carried a cyst. Unidentified black parasitic cysts were found on 13 individuals, 10 of which were from St Andrews Drain. Red parasites, possibly nematodes, were found on 11 individuals, 7 of which were from Te Roto Repo o Tawera. The only parasite observed at Dog Kennel Stream was *I. multifiliis*, and then only at low prevalence and intensity. In total, *N. burrowsius* individuals generally only harboured a single cyst, although some were heavily infected (Figure 6.3). Across sites the intensity of *I. multifiliis* infection mirrored the pattern in the prevalence of infection. Mean (± 1 SE) numbers of *I. multifiliis* cysts per individual for sites in order of prevalence were 4.8 (0.6), 3.1 (1.1), 1.3 (0.2), and 1.2 (0.2), for Hororata Spring, St Andrews Drain, Te Roto Repo o Tawera, and Dog Kennel Stream, respectively.

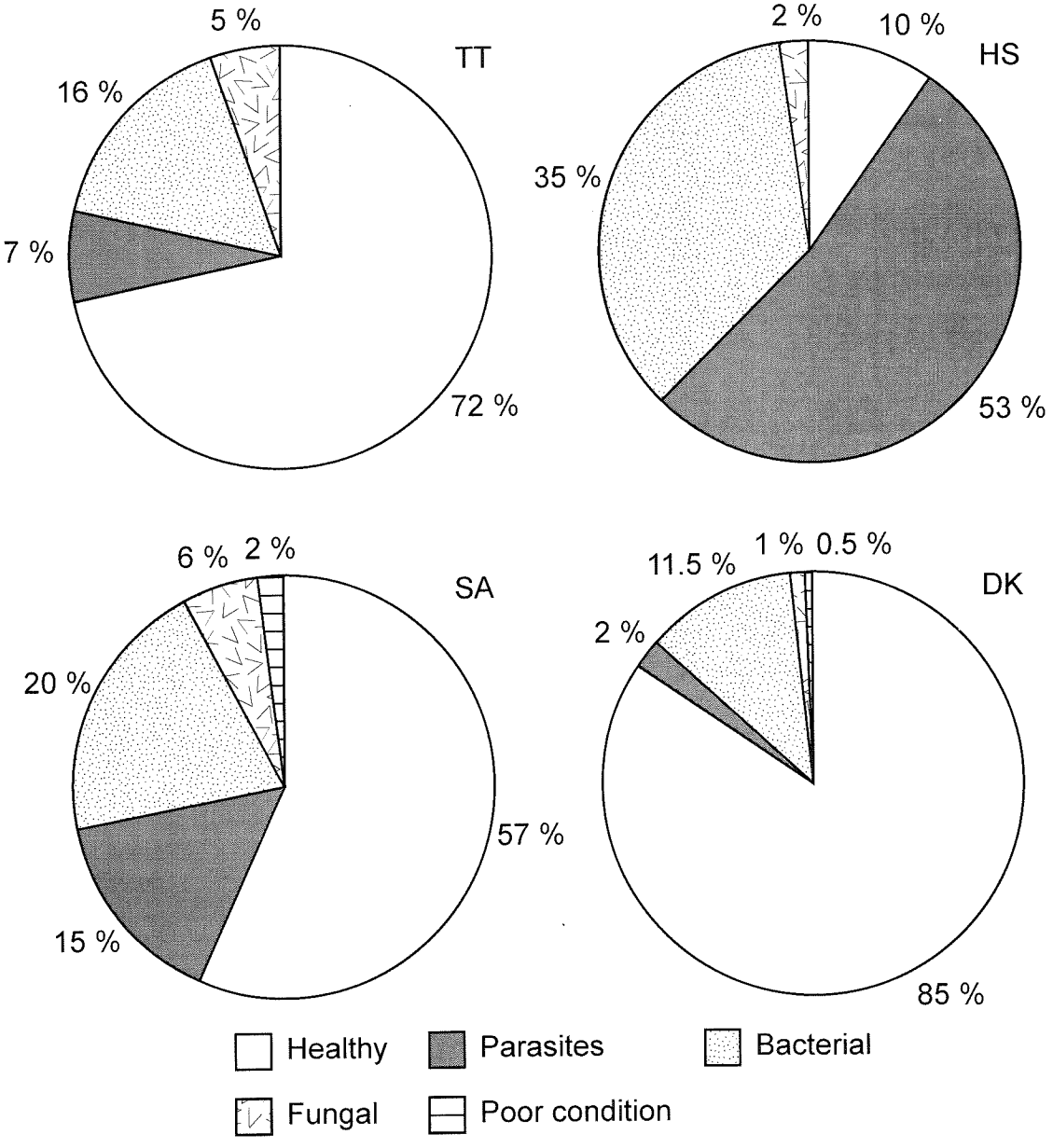

Figure 6.2. Prevalence of observed infection and disease in *N. burrowsius* populations at each site. TT = Te Roto Repo o Tawera, HS = Hororata Spring, SA = St Andrews Drain, and DK = Dog Kennel Stream.

Table 6.1. Location of bacterial infection and finrot observed on *N. burrowsius* at all sites combined as percentage occurrence.

Location	Bacterial infection	Finrot damage
Head	4	
Jaw	9	
Side	8	
Pectoral fin	8	2
Caudal peduncle	6	
Dorsal fin	4	9
Caudal fin	42	84
Anal fin	37	9
Pelvic fin	3	

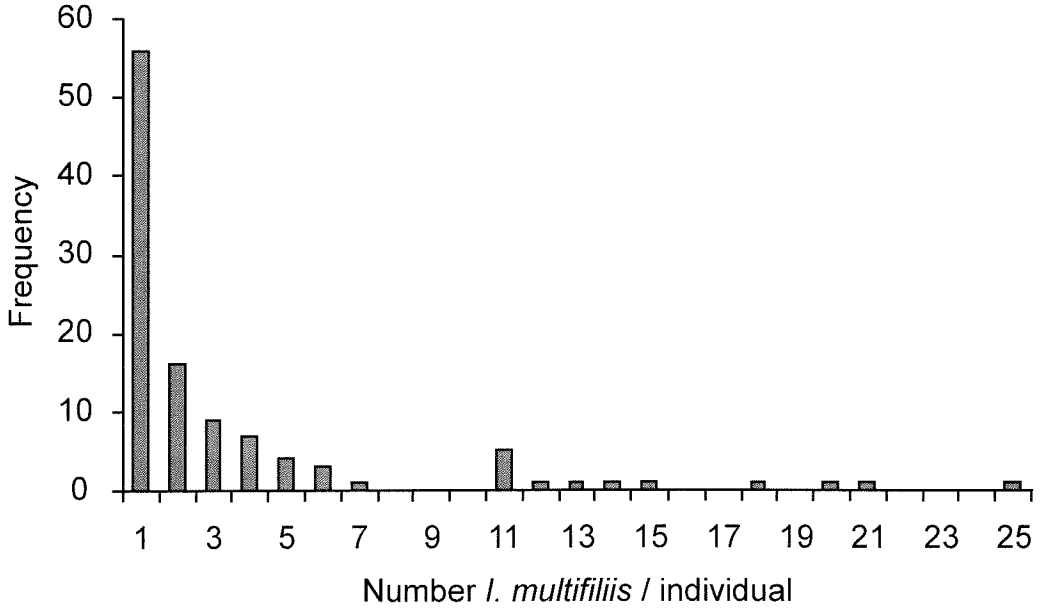

Figure 6.3. Intensity of parasitic infection indicated by the number of *I. multifiliis* cysts per *N. burrowsius* individual in all four sites combined.

Incidence of deformity

Fifteen of the 1091 *N. burrowsius* examined in May 2002 were observed to have grossly visible signs of deformity. The highest percentage of examined *N. burrowsius* with deformity was found at Dog Kennel Stream (Table 6.2), whereas, St Andrews Drain contained the lowest. Deformities predominantly involved characteristic changes to fins, typically their reduction, contorted malformation and/or absence of fin rays. This broadly similar deformity most commonly involved the pectoral and caudal fins and was found at all sites, though most prevalently at Te Roto Repo o Tawera. The abnormal fins were often difficult to spread due to thickening of the skin, and possibly cartilage, and they also often had a distinctly pointed and upturned shape. Malformations were also seen in dorsal and anal fins, which involved reduced and missing fin rays. There were no observed cases of deformed pelvic fins. This may have been due to the already reduced state of these fins in *N. burrowsius* making deformity difficult to detect.

The second most common deformity involved the partial or complete reduction of the opercular region. Typically the gills on one side were partially or totally exposed, being most severe at Dog Kennel Stream. Other deformities observed involved the curvature of the spine or caudal region. In one case, a swim bladder and/or spinal deformity forced a fish to swim upside down; however it was in surprisingly good condition. Individuals with extreme deformities, such as exposed gills or curved spines, tended to be 0+, suggesting that long-term survival with such disabilities is questionable. However, large adults with deformed fins were also found. In addition to deformities, injuries were also noted, but were not included in deformity comparisons. The most common and severe injury was to the tail region, most likely a result of an attack by white faced herons (*Ardea novaehollandiae*), and was observed at St Andrews Drain, in particular. *A. novaehollandiae* are implicated as the injuries appeared to be healed wounds or recent and infected injuries, strongly suggesting the region had been bitten off. In one case the entire tail region and much of the caudal peduncle was missing. Injury and mortality due to *A. novaehollandiae* has also been observed at Te Roto Repo o Tawera. These injuries could not have been caused by shortfin eels (*Anguilla australis*), as they did not occur at these sites.

Table 6.2. Summary of deformities found in examined fish from four sites; where TT = Te Roto Repo o Tawera, HS = Hororata Spring, SA = St Andrews Drain, and DK = Dog Kennel Stream.

Site	Reduction of structures					Other		# examined	% deformity
	Operculum	Fins				Spine curvature	Swim bladder		
		Pectoral	Dorsal	Anal	Caudal				
TT		2		2	2			374	1.6
HS					1			61	1.6
SA	1	1				1	1	442	0.9
DK	2		1			1		214	1.9

Association between fish health and environmental variables

Correspondence analysis was used to summarise the associations between *N. burrowsius* populations, characteristics of their health, and environmental conditions (Figure 6.4; Table 6.3). Dimension one of this analysis explained 56.3 % of the variation, and dimension two 31.4 %. In this analysis the proximity of variables to one another indicates the level of correspondence. Dimension one associated the occurrence of deformity with the environmental variables: water temperature, dissolved oxygen, nitrates, and phosphates. Whereas, the incidence of fungal infection was most closely associated with pH, and flow, across dimension one. Bacterial and parasite infections were not closely associated with any environmental variable on dimension one. However, dimension two associated these pathogens with water temperature and the presence of grazing. A further interesting association across dimension two was between percentage health and deformity, as observed at Dog Kennel Stream and Te Roto Repo o Tawera. This may indicate that deformed individuals have higher survival likelihood in the absence of pathogenic infection.

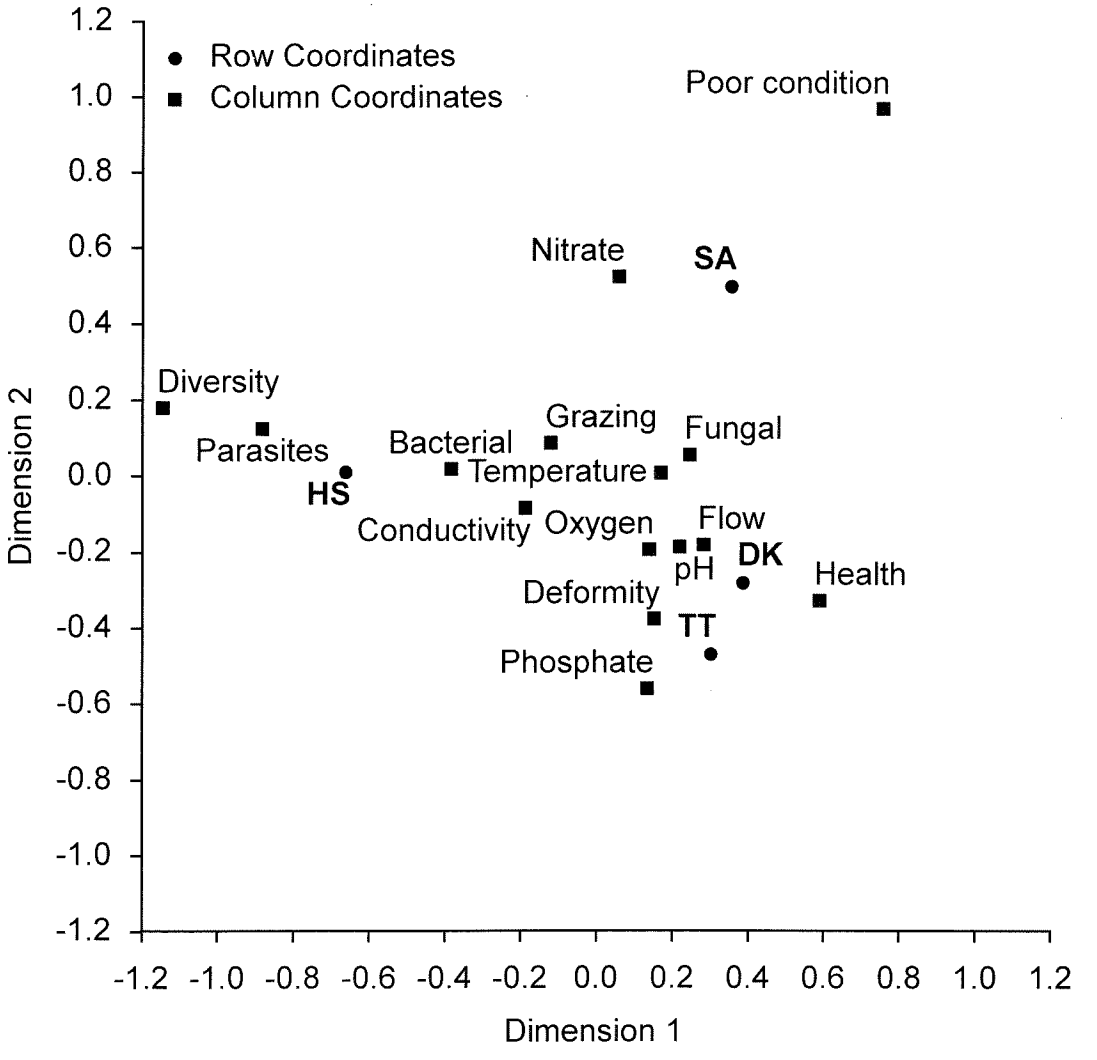

Figure 6.4. Correspondence analysis showing associations, across all sites, between variables of population health and water quality (pH, phosphate (mg/l), Conductivity (µS), dissolved oxygen (percentage of air saturation)). Grazing and flow were rank variables. Study sites are in bold, where: TT = Te Roto Repo o Tawera, HS = Hororata Spring, SA = St Andrews Drain, and DK = Dog Kennel Stream.

Table 6.3. Mean water chemistry parameters measured in April and May 2005, one month before and during *N. burrowsius* examination for pathogenic infection.

	Tawera	Hororata	St Andrews	Dog Kennel
Nitrate (mg/l)	0.13	0.48	0.75	0.14
Phosphate (mg/l)	8.50	6.36	2.12	6.18
% dissolved oxygen	93.0	69.9	67.1	29.4
Conductivity (µS)	307.2	147.8	597.9	955.8
pH	6.8	6.5	6.7	6.9
Temperature	10.9	11.8	13.9	10.0

DISCUSSION

In this study, I found that the most prevalent forms of pathogenic infection in *N. burrowsius* were bacterial hemorrhagic septicaemia and *I. multifiliis*. Decreased genetic diversity may increase the prevalence of pathogens in a population because of shared susceptibilities and deleterious genetic factors. For example, high genetic heterozygosity has been linked to resistance to *I. multifiliis* parasitism in rainbow fish (*Melanotaenia* spp.; Gleeson et al. 2000). However, the sites with no detectable nucleotide diversity (Te Roto Repo o Tawera and Dog Kennel Stream; Davey et al. 2003) were associated with higher levels of apparent *N. burrowsius* health. Thus, extrinsic factors likely underlie the observed significant differences in health between *N. burrowsius* populations.

The population that appeared most afflicted by parasitic and bacterial infection was Hororata Spring. The high level of agricultural and hydrological disturbance experienced by this *N. burrowsius* population may have contributed to their poor health, likely exacerbated by the presence of large numbers of waterfowl. The presence of water fowl can result in increased numbers of bacteria in the water and substratum, through droppings (Snieszko 1974). Stock faeces also contribute substantial amounts of bacteria to receiving waters (Hickey et al. 1989). Thus, it is possible that increased exposure to bacteria resulted in the greater prevalence of bacterial infection in *N. burrowsius* at Hororata Spring. Similarly, *N. burrowsius* at St Andrews Drain had a high rate of bacterial hemorrhagic septicaemia. This site is hydrologically stable, however it experiences considerable agricultural disturbance (Chapter 4). Thus, the presence of stock, and their access to, and defecation in, the waterway may be a more important factor in the prevalence of bacterial infection, than stress induced by hydrological disturbance.

Ichthyophthirius multifiliis

The ubiquitous ecto-parasite *I. multifiliis* is one of the most dangerous parasites to fish (Hoffman 1973), and can cause large scale mortality in wild populations (Wurtsbaugh & Tapia 1988; McDowall 1990). Due to its global distribution and high fatality rates, *I. multifiliis* has been extensively studied (Hoffman 1973). There are several predictions concerning the occurrence of *I. multifiliis*, including that it is more prevalent in still, warm water (McDowall 1990), and can rapidly multiply as temperatures increase (Schubert 1987). Conversely, this protozoan is suppressed by water velocities above 85 cm/min (Bodensteiner

et al. 2000), low dissolved oxygen levels (van Duijn 1973), and a pH less than 5.5 (Schubert 1987). It has been suggested that benthic feeding fish are more vulnerable than pelagic species, because the *I. multifiliis* life cycle includes a benthic cyst (tomont), from which the mobile infective stages (swarmers or theronts), develop (Wurtsbaugh & Tapia 1988). The prevalence of *I. multifiliis* infection will be dependent on the density of the fish community, as swarmers require contact with a host within 48 hours (Schubert 1987). Furthermore, mature parasites (tomont), require a stable surface, such as aquatic vegetation or cobbles, on which to attach as cysts while they divide into theronts. Thus, the condition of habitat and water quality at each site is likely to have influenced the prevalence of *I. multifiliis*. Water temperatures measured at Hororata Spring and St Andrews Drain were higher than other sites, potentially explaining higher prevalence rates, via increasing generational turn over. Relatively high water velocity at Te Roto Repo o Tawera is likely to explain low *I. multifiliis* incidence at this site, despite the high density of *N. burrowsius* (Chapter 5). In comparison, *I. multifiliis* appeared to be limited by low dissolved oxygen levels at Dog Kennel Stream. Interestingly, although dissolved oxygen was nearly absent in some of the deeper pools at Dog Kennel Stream, any stress that this may have placed on individuals was not translated into an increased incidence of disease, as initially hypothesised. Thus, a population may be protected if a host species can tolerate more severe environmental conditions than its parasites and pathogens.

It is difficult to assess the impact of parasitism in the field, especially for small fish, as mortality levels are difficult to determine (Lopukhina et al. 1973). Although the prevalence of *I. multifiliis* was high at some sites, the intensity of infection may have been sufficiently low, so as not to pose a risk of direct mortality. In a documented mass mortality of an estimated 18 million killifish (*Orestias* spp.), through heavy infestation of *I. multifiliis*, one 140 mm *O. agassii* hosted more than 1800 protozoa (Wurtsbaugh & Tapia 1988). However, this is an extreme example, as it followed the introduction of the parasite into a naïve community (Wurtsbaugh & Tapia 1988). In contrast, Lopukhina et al. (1973) found that 6 – 12 protozoa per individual appeared to cause mortality in 25 – 27 mm fry of ide (*Leuciscus idus*) and 18 – 20 mm fry of roach (*Rutilus rutilus*). Thus, it is likely that *N. burrowsius* individuals hosting 20 – 25 protozoa would have experienced some disadvantage. This is supported by the observation of a heavily parasitised *N. burrowsius* foraging, while being visually obvious during the day at Hororata Spring in 1999, which was unusual for this largely nocturnal species. It has been demonstrated that to compensate for higher nutritional requirements, parasitised fish forage more actively and recover more quickly after a

frightening stimulus, compared with non-parasitised fish (Giles 1987). This change in behaviour can make fish more susceptible to predation (Poulin 1993). Thus, a high parasite loading in the presence of an opportunistic predator, such as A. australis is likely to adversely affect *N. burrowsius* populations at Hororata Spring.

Deformity

It was hypothesised that deformity would be highest in *N. burrowsius* populations with the lowest genetic diversity. This result was supported in three out of four populations. That is, *N. burrowsius* from Dog Kennel Stream having no detectable nucleotide diversity (Davey et al. 2003), had the highest incidence of deformity; and in fish at Te Roto Repo o Tawera, also with no nucleotide diversity, a consistent pattern of fin reduction deformity was observed. In contrast, *N. burrowsius* in St Andrews Drain had the lowest occurrence of deformity and sampling has indicated that genetic diversity is relatively high compared to other *N. burrowsius* sites (Davey et al. 2003). The exception was Hororata Spring, having the highest nucleotide diversity of the study sites examined, and moderate rates of deformity. However, the proportion of deformity calculated for Hororata Spring could also be biased due to the small sample size examined.

The level of deformity found in this study is higher than levels found in some other studies conducted on wild fish populations. Patten (1968) found that 0.24 % of freshwater fish in the polluted Washington area were deformed, whereas, in an unmodified estuary only 0.06 % of the fish community demonstrated any abnormity (as cited in Dahlberg 1970). Nonetheless, in New Zealand, Allibone (2000) found the occurrence of deformity involving the head, jaw structure, and occasionally reduced fin development, ranged up to 19 % in a study of non-migratory galaxiids in Otago. However, this was likely due to deleterious effects of hybridisation between two closely related species. Much literature on fish deformities originates from aquaculture, as deformities are more prevalent in hatchery reared fish than wild populations (Snieszko 1974; Sadler et al. 2001; Beraldo et al. 2003). This is largely due to the absence of natural selection pressures, and a lack of predators and competitors, which may otherwise cause high mortality in deformed individuals (Dahlberg 1970; Sadler et al. 2001). Due to this mortality it is difficult to assess absolute rates of deformity in wild populations (Lopukhina et al. 1973). Furthermore, separating genetic and environmental factors is complex as environmental conditions may exacerbate underlying genetic susceptibilities (Sadler et al. 2001). However, the prevalence of certain types of deformity

can be informative. For example, skeletal deformities are considered to be influenced by the genetic characteristics of individuals (Sadler et al. 2001). Whereas, asymmetrical deformities are considered non-inheritable (Beraldo et al. 2003). Deformities of the fin are common in fish and it is suggested that their formation has a genetic basis. One widespread example is the complete or partial abnormal absence of pelvic fins (Alvarez-León 1980). Malformation may persist in populations as the pelvic fins are functionally the least important fins (Alvarez-León 1980). Some populations also exhibit malformations that include extra fins (Heithaus & Laushman 1997). In a survey of Loch Lomond, Brown & Scott (1987) found 1.5 % of the Scottish powan (*Coregonus lavaretus*) sampled to have an extra pelvic fin. In New Zealand, specimens with reduced or absent fins were described by Stokell (1938, 1940) for upland longjaw (*Galaxias prognathus*), and alpine galaxias (*G. paucispondylus*). Moreover, fin deformities have been commonly noted in *Neochanna* species, including *N. apoda* and *N. burrowsius* (Eldon 1978, et al. 1978). In studying black mudfish (*Neochanna diversus*), McPhail (1999) observed reduced pectoral ray counts, with 28 % of fish having counts below the minimum stated by McDowall (1970). Deformities involving reduced fins may not be unexpected considering anal fins are already vestigal or entirely missing in several species of *Neochanna* (McDowall 1997a).

Defects in the opercular complex, including reduction, are also common in many fish species. Such deformities can negatively affect biological functions, e.g. respiration is impaired due to reduced efficiency of the buccal pump, through reduced mechanical efficiency of the operculum; also exposed gills are more susceptible to physical damage, oxygen stress, and infection by pathogens (Beraldo et al. 2003). In my study *N. burrowsius* at Dog Kennel Stream exhibited severe asymmetric operculum deformity, which is considered non-inheritable, but may also indicate an underlying genetic susceptibility in this population. The opercular structure is fragile and easily alterable by external agents during early development stages. A plethora of causes for this deformity have been suggested, including nutritional deficiencies, vitamin-C deficiency, heavy-metal contamination, and insufficient supply of essential fatty acids or vitamins (Sadler et al. 2001; Beraldo et al. 2003). In the present study, it is interesting that opercular deformity in *N. burrowsius* was highest at the site with the lowest dissolved oxygen levels. Thus, gill cover reduction could possibly be beneficial during periods of hypoxia by allowing greater, more direct, oxygen absorption from the water. The high level of operculum deformity at Dog Kennel Stream may also be attributed to low dissolved oxygen during egg development.

Conclusion

In my study, extrinsic factors were related to pathogenic infection rates. Bacterial infection was likely related to the level of agricultural and the presence of waterfowl, and to a lesser degree hydrological disturbance. *N. burrowsius* populations with high rates of bacterial infection also had high *I. multifiliis* loadings. However, this may be related to circumstantial environmental conditions, as well as, agricultural or hydrological disturbances. Both intrinsic and extrinsic factors are likely to underlie the patterns of deformity observed. However, deformities in *N. burrowsius*, particularly fin malformation suggest inherited deformity in the study populations. Furthermore, there is preliminary evidence that populations with the lowest genetic diversity had the highest rate of deformity. However, further studies are required to determine the extent that genetic and environmental factors contribute to the observed patterns in deformity. Understanding the degree to which deformities translate into reduced fitness and the link between deformity and *N. burrowsius*' low genetic diversity will provide information of importance to *N. burrowsius* conservation efforts.

SECTION 3

Abiotic and biotic factors

At top an upland bully looks down on a Canterbury mudfish. At bottom footprints of herons and mustelids ring the edge of a drying pool at Hororata Spring.

Chapter 7.

Size-dependant strategies in response to hypoxia and drought by *Neochanna burrowsius*.

INTRODUCTION

Disturbance can leave a lasting imprint on community composition, population-structure, and species traits (Smale & Rabeni 1995). This is because the nature and intensity of disturbance can result in differential mortality risk related to the size and behavioural response of individuals. In many small aquatic systems, seasonal episodes of drought disturbance occur. Such droughts develop slowly, with the aquatic habitat advancing through a process of flow cessation, progressive hypoxia and finally habitat desiccation (Lake 2000). During this process, habitat can become fragmented and aquatic life confined to remnant pools. This can lead to the intensification of biotic interactions, and coupled with stressful abiotic conditions may result in high mortality (Power et al. 1988; Resh et al. 1988). However, in such situations, individuals may not be equally vulnerable to mortality.

Fluctuations in water level, with subsequent hypoxia, and habitat desiccation, are a common feature of wetlands (Partridge 1982; Buxton 1991). As a result, many wetland species have evolved behavioural and physiological mechanisms to survive sustained periods of drought. Canterbury mudfish (*Neochanna burrowsius*), the focus of this study, is a wetland specialist exhibiting behavioural responses to low dissolved oxygen levels that are likely to enhance its survival during drought (Eldon 1979a). In the laboratory, three behavioural responses to hypoxia were observed in *N. burrowsius* by Meredith (1981). He found the most common behaviour was that fish rose to the surface, took and held an air bubble in their buccal cavity, then returned to the bottom of the tank and performed 'burrowing like' activities. Meredith (1981) postulated that this behaviour was an attempt to lodge themselves under an object to prevent subsequent ascent to the surface. Some fish however, did not seek cover and remained at the water surface, using the air bubble in their buccal cavity for buoyancy. These fish did not expend as much energy as fish exhibiting the former behaviour. The final behaviour was for fish to attempt to leave hypoxic water, without establishing a pattern of air breathing (Meredith 1981). Collectively these three behaviours indicate that *N. burrowsius*

exhibit a variety of responses to hypoxia in the laboratory. In this study, I investigated behavioural responses of *N. burrowsius* to hypoxia in the field.

N. burrowsius may also actively leave the water to avoid low dissolved oxygen levels (Meredith 1981). Certainly, *N. burrowsius* show an ability to survive without water (emersion) for considerable periods, likely to be between one and five months (Eldon et al. 1978; Meredith 1985). During droughts, *N. burrowsius* have been found in vegetative debris, root masses, car tyres, and burrow-like structures (Phillipps 1926; Eldon et al. 1978; Eldon 1979a). The occurrence of *N. burrowsius* in burrows has attracted considerable popular attention and scientific debate (Eldon 1979c, 1985; McDowall 1990, 1998; Swales 1991; Gray 2000). However, there is uncertainty surrounding the ability of *N. burrowsius* to create these burrows, primarily because fish did not attempt to avoid desiccation by burrowing under controlled conditions (Eldon 1979c). This implies that *N. burrowsius* may sometimes become stranded as water levels recede, and those fish that do not find suitable refuge may perish (Hartman 1990). Even those *N. burrowsius* found in holes and burrows can suffer substantial mortality, with drought disturbance capable of drastically reducing *N. burrowsius* populations (Eldon et al. 1978). In general, survival is likely to be dependent on microhabitat characteristics, such as moisture and cool temperatures (Meredith 1985). Furthermore, *N. burrowsius* is able to survive emersion because of a low metabolic rate, cutaneous respiration, and tolerance of homeostasis imbalance (Meredith 1985). Such amphibious adaptations, coupled with high vagility and fecundity, may facilitate persistence in seasonally dry habitats (Eldon et al. 1978; Meredith 1985; Poff & Ward 1989). It has also been suggested that *Neochanna* spp. exhibit the ability to aestivate when emersed however, this has been debated and is unlikely to be the case in *N. burrowsius* (Meredith 1985; O'Brien & Dunn in press).

N. burrowsius populations that frequently experienced periods of hypoxia and habitat desiccation differed in size-structure to those in habitats that perennially flowed (Chapter 5). Meredith (1985) proposed the concept of an optimal survival size, based on constraints imposed by drought, to explain the general small size of *Neochanna* species. He suggested the presence of an upper and lower size limit that would determine behavioural responses and survival success. This theory was based on physiological constraints, relating to body size, having ecological consequences for fish experiencing progressive hypoxia and emersion. Size influences energy reserves because there is a negative relationship between body size and metabolic rate (Cargnelli & Gross 1997). Furthermore, the surface area to volume ratio of fish controls the efficiency of oxygen absorption and the rate of desiccation. Thus, smaller

fish are expected to be more tolerant of hypoxic conditions, while larger fish are likely to survive emersion for longer periods (Burleson et al. 2001). In this study, I investigated whether deteriorating environmental conditions apply size-dependent constraints on *N. burrowsius*, leading to differing behavioural strategies of drought survival. Further, I describe field observations of unusual shoaling behaviour during periods of hypoxia, and aggregation during habitat desiccation.

METHODS

This study was conducted at Hororata Spring (see Chapter 1 for site description), which experienced annual periods of hypoxia and drying during late summer and autumn. Periodic observations of *N. burrowsius* behaviour, and sampling of habitat use, were conducted during summer and autumn from 1999 – 2001. When water was present, sampling by Gee minnow trapping and electrofishing was conducted to determine *N. burrowsius* abundance (see Chapter 2 for further sampling methods). When pools were dry, the substratum was searched for the presence of fish. Any potential holes were excavated by hand, and sections of fallen and standing bank were broken up during searches. However, these searches were not extensive due to their destructive nature. Where possible, dissolved oxygen content (measured with an YSI Model 95 DO meter), and conductivity (measured with an Oakton pH/CON 10 meter) of the water present were recorded.

Live fish were anesthetised and handled as described in Chapter 2, and measured as described in Chapter 5. Live *N. burrowsius* found in subterranean refuge were replaced as found, covered with cobbles, and water was poured over the area to replace any lost moisture. Dead fish were measured where possible and preserved in 70 % ethanol.

Comparison of immersed and emersed *N. burrowsius*

During May 2001, Hororata Spring was extremely dry, with only small remnant pools remaining. Indeed, during autumn 2001 the Canterbury Plains only received 40 – 70 % of its average annual rainfall, and in some places the lowest ever rainfall was recorded (Aitchison-Earl et al. 2001). This provided the opportunity to compare the size and condition of emersed fish, with fish still immersed in a remnant pool. The location where emersed fish were found was connected, to the deep permanent pool (Figure 7.1) during high flows. In this study, the permanent pool was named Spring Pool and the ephemeral pool the Confluence (see Chapter 1). At the time of sampling there was approximately 10 m of dry habitat separating the

Spring Pool and the lowest point of the Confluence, where emersed fish were found. The permanent Spring Pool was electrofished, whereas emersed *N. burrowsius* were dug by hand out of the substratum.

Figure 7.1. Spring Pool habitat at Hororata Spring during May 2001, showing extent of drying. The usual water level is indicated by the vegetated ridge above the bare cobble substratum.

Statistical analysis

Relative condition (Kn) was calculated using the power equation of the length – weight relationship obtained from immersed and emersed fish combined, and applied to each fish using the equation $Kn = Weight / 2.0^{-6} \cdot Length^{3.23}$. When comparing relative conditions a Kn value of 1 signifies a fish with average condition (see Chapter 5 for further details). Differences in logarithmically-transformed data of length and relative condition between fish were tested by ANOVA using Statistica 6.0 (Statsoft Inc. 2001).

RESULTS

Shoaling behaviour

During late summer 1999, Hororata Spring had dried to a series of pools and observations of atypical *N. burrowsius* behaviour were made. *N. burrowsius* are usually nocturnal, benthic and solitary (Eldon 1979a). However, during this period, in the large shaded Willow Pool (Chapter 1), *N. burrowsius* were observed, during the day, swimming in large groups (> 100 individuals of various sizes) at the water surface. These were predominantly loosely co-ordinated shoals; however, more co-ordinated schooling behaviour was observed when fish were startled. *N. burrowsius* were also observed amongst thick curtains of adventitious aquatic crack willow (*Salix fragilis*) roots at the water surface, an activity that formed many small holes in the willow roots. Eldon (1979c) also observed such holes and described how the adventitious roots were sometimes honeycombed with holes. However, he never documented *N. burrowsius* using these holes. Shortfin eel (*Anguilla australis*) were also seen foraging during the day, stalking *N. burrowsius* in the water column and positioning themselves amongst willow roots watching the passing shoals of fish. One large *A. australis* was found dead, but no other dead fish were found. Minimum and maximum temperatures for a 24 hour period including these observations were 12.5 °C and 26 °C respectively. Daytime dissolved oxygen readings during this period were 6.7 ± 1.2 mg/l, (mean ± 1 SE) with a ~ 2 mg/l difference between the surface and bottom of the pool.

Incidence of stranding

On 10 May 2000 an isolated pool was found that appeared to have recently dried. This was indicated by the moist mud substratum, and a large number of stranded, but alive, aquatic invertebrates. The habitat was searched and two *N. burrowsius* were located. The first (60 mm TL) was dead and desiccated on the substratum surface at the deepest point of the pool. The second (54 mm TL) was alive, buried 10 cm deep, under a large cobble, at the interface of the surface mud layer and a gravel sub-horizon. These observations indicate that *N. burrowsius* can become stranded and may quickly desiccate without suitable refuge.

Occurrence in burrow

During May 2001, two *N. burrowsius* were found within clumps of eroded stream bank, in a desiccated area of the Confluence habitat. One *N. burrowsius* was in a burrow, 38.6 cm in length, with a diameter of 12 mm at the end, and 13 mm at its widest (Figure 7.2). The burrow entrance was 1.7 m below the level of bank full discharge. Moisture was still present

in the far end of the burrow and the fish was immediately active. The presence of red-orange staining on the inside surface of the tunnel suggested that the iron oxide had developed *in situ*, indicating that this burrow was not recently constructed and was likely to have been filled with oxygenated water (Figure 7.2).

Figure 7.2. Cross section of burrow containing *N. burrowsius*.

Occurrence in chamber

A large number of *N. burrowsius* (> 100) were found below the substratum surface of the dry Confluence habitat in May 2001. Although many *N. burrowsius* were in fine sediment within the interstitial spaces of cobbles, most were in a single large chamber approximately 40 cm below the substratum surface. The discovery was made after a small crack in the mud, at the base of the stream bank was excavated by hand to a cavity 55 x 36 cm, and to a depth of 52 cm (Figure 7.3). The excavated substratum was moist; however, the large chamber was 10 cm above the water table. This groundwater had a temperature of 12.2 °C and contained 2.9 mg/l dissolved oxygen.

Live, emersed *N. burrowsius* in this chamber reacted when disturbed, especially the smaller fish, however some were very sluggish. Many were lying on their dorsal surface, assuming a

position previously described by Meredith (1985). Innumerable *N. burrowsius* skeletons were also in the chamber, forming a mass of scattered bones. This implied multi-year use as it was unlikely, considering the state of their decomposition that so many skeletons could accumulate in one season. However, some fish appeared recently dead; being partially decomposed and covered with very small fly maggots. These flies must have followed the fish underground and were also noted by Eldon et al. (1978) in similar situations. These recently dead fish were not desiccated and it is possible that the activity of the maggots could have hastened the mortality of the immobile fish.

During excavation *N. burrowsius* were found 15 – 42 cm below the substratum surface. Mortality appeared to be related to the location and substrate type these *N. burrowsius* were found in (Figure 7.4). Of the *N. burrowsius* found between 15 – 26 cm deep in predominantly cobble and silt substratum, 65 were alive and 3 dead. Below this depth, a distinct dark horizon of clay was present and *N. burrowsius* showed higher mortality (24 alive, 31 dead) than in sandy deposits below (15 alive, 4 dead). Mortality rate was not independent of location and substratum type ($\chi^2 = 42.3$, df = 5, $p < 0.001$). Thus, characteristics of the underlying substratum affected mortality rates of *N. burrowsius* during emersion.

Figure 7.3. Distinctive banding of substratum in which *N. burrowsius* were found emersed. A dark horizon of clay between layers of lighter sandy substratum is pointed to.

Figure 7.4. Chamber containing numerous *N. burrowsius* found below the substratum surface in a habitat that had dried up.

Comparison of emersed and immersed fish

The length and weight of 65 *N. burrowsius* captured in the permanent Spring Pool and 104 live fish found emersed were measured. Of the dead emersed fish, 28 were sufficiently intact to allow measurement of their length. There was a significant difference (ANOVA: $F_{(2, 194)} = 17.85$, $p < 0.001$) between the lengths of emersed and immersed *N. burrowsius* (Figure 7.5), with *N. burrowsius* found emersed being significantly longer than fish found freely swimming in an adjacent remnant pool. Furthermore, fish that had emersed themselves, but had suffered mortality, were significantly shorter than emersed fish which were still alive when found. Thus, it appears that there is an upper limit to the size of fish capable of remaining immersed and a lower size-limit related to emersion survival. Generally, *N. burrowsius* > 50 mm TL adopted a strategy of emersion and *N. burrowsius* shorter than this remained immersed.

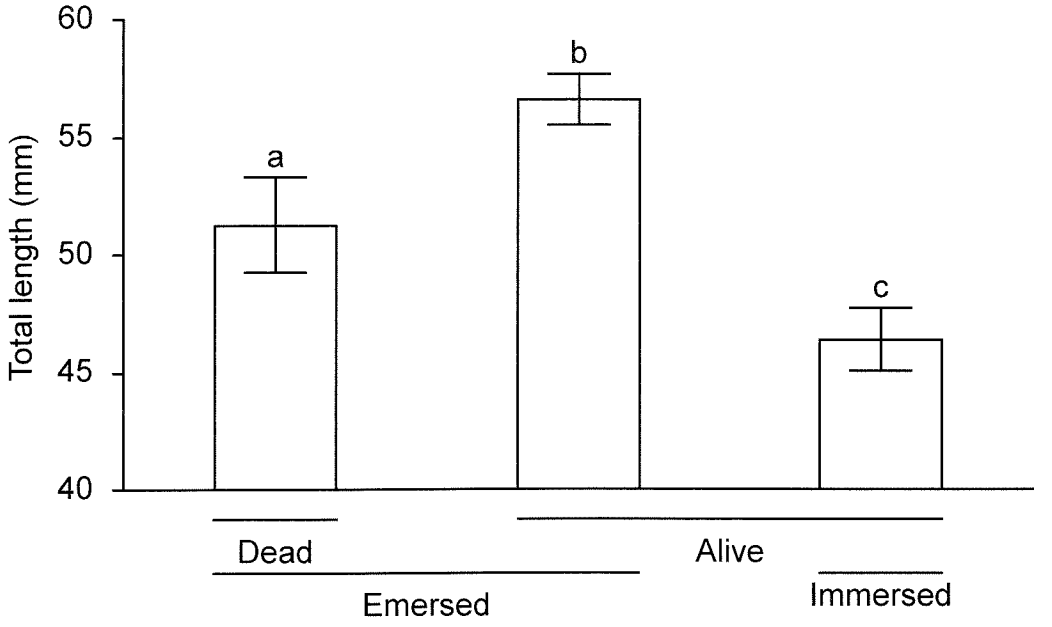

Figure 7.5. Length (mean ± 1 SE) of *N. burrowsius* employing differing strategies during a drought. Emersed fish were found buried in gravel at the base of a stream bank, either dead or alive. Immersed fish were live fish electrofished from an adjacent pool. Different letters indicate significant differences as determined by Tukey post hoc tests.

Emersed fish had the same mean relative condition (Kn) as free swimming immersed fish (ANOVA: $F_{(1, 167)} = 0.013$, $p = 0.91$; mean for both groups, Kn = 0.89), which indicates that many fish in both situations had generally poor condition. However, the relative condition of emersed fish was less variable (range = 1.3 – 0.43, SE = 0.03) compared with free swimming immersed fish (range = 1.97 – 0.35, SE = 0.07).

The Confluence habitat also dried up in 1999. Thorough sweep netting of the pool was conducted just prior to it totally drying, when the extent of water was 4.1 x 3 m with a maximum depth of 0.12 m. Eight *N. burrowsius* were captured with sizes ranging from 45 – 54 mm TL, averaging 49 mm TL. No other fish were found in this pool, which dried subsequently, but the area was not searched further. During this period, the Spring Pool had dried to approximately half of its usual volume. Eight *N. burrowsius* were also caught in this habitat by sweep netting. These fish ranged from 39 – 55 mm TL, averaging 47 mm TL. There were no significant differences in the length or weight of fish between the Confluence and Spring Pool habitats during this survey.

DISCUSSION

The behaviours of shoaling and seeking refuge in the substratum during drought were first recorded in *N. burrowsius* by Mr A. Burrows of Oxford whom the species is named after. He recounted in a letter to W. J. Phillipps of the then Dominion Museum in Wellington that 'fish...4 in. to 6 in. in length...swim in small shoals. The waterhole became dry... [and] I found it in holes shaped like a coconut in which the fish hid, each with a small entrance. The holes were very smooth inside' (Phillipps 1926, p. 532). However, adult *N. burrowsius* only exhibit facultative shoaling and no other reference to this behaviour occurs in the literature.

Shoaling

There are likely to be several factors, relating to drought processes that compel *N. burrowsius* to form large aggregations during the day at the water surface, which is a risky behaviour. In particular, reduced dissolved oxygen levels and food resources, and increased predation risk may all result in an increased tendency to shoal. The increased activity of *N. burrowsius* during the day is unusual and could to be related to a reduction in food resources compelling individuals to extend foraging to maintain energy resources. Grand & Dill (1999) determined experimentally that with greater shoal size, increases in risk-taking behaviour occurred as a consequence of increased competition for scarce resources. Drought imposes resource limitations on fish due to deteriorating environmental conditions affecting prey, and increased competition in a confined habitat (Magoulick & Kobza 2003). Additionally, greater food resources may be required at the elevated water temperatures associated with droughts because of increased metabolic rates. Food limitation may also explain why so many fish were congregating in the Willow Pond. The presence of trees provided shade, which may have reduced water temperatures. Furthermore, it is likely that the dense overhanging canopy was a source of falling terrestrial insects, being taken at the water surface. The presence of terrestrial items in gut contents of *N. burrowsius* during this period supports this suggestion (Chapter 8).

Low dissolved oxygen levels will also necessitate fish to utilise the water surface. However, levels measured during this period at Hororata Spring were not as low as has been measured at other habitats where shoaling has not been observed. Nonetheless, in laboratory situations, *N. burrowsius* responded to very low dissolved oxygen levels by moving to the water surface to gulp air or utilise the oxygen rich surface layer (Eldon 1979c; Meredith 1985). Thus, while drought-induced resource limitation and hypoxic stress are likely explanations of diurnal and

pelagic activity by *N. burrowsius*, they do not fully explain the progression to shoaling behaviour. Furthermore, in habitually shoaling herrings (*Clupea harengus*), Domenici et al. (2002) reported that school volume increased with decreasing dissolved oxygen, rather than eliciting stronger schooling behaviour.

Air breathing at the water surface increases the risk of predation by both aerial and aquatic predators (Lima & Dill 1990). In fish species, shoaling is an advantageous anti-predator strategy and the presence of a predator can act to concentrate schools of fish (Lima & Dill 1990). The presence of a predator can also influence air breathing behaviour in fish. Dwarf gouramis (*Colisa lalia*) reduced surface air breathing in the presence of the air breathing predatory snakehead (*Channa micropeltes*; Wolf & Kramer 1987). More interestingly, the presence of a predator has been shown to increase the synchronicity of air breathing, which is likely to dilute predator risk (Kramer & Graham 1976; Gee 1980; Lima & Dill 1990). Such anti-predator benefits of synchronised behaviour may lead to shoaling in *N. burrowsius* during periods of low dissolved oxygen and the presence of actively foraging *A. australis*.

Shoaling behaviour and the discovery of a large number of *N. burrowsius* utilising an underground chamber, in desiccated habitat, may be linked. Suitable refuge during drought may be scarce, and survival a matter of chance (Eldon 1979a). An interesting possibility is that by congregating, and co-ordinating their movement as water becomes hypoxic, *N. burrowsius* may be able to follow one another into suitable refuge, possibly lead by experienced individuals, thus increasing the likelihood of finding refuge. Social learning has previously been demonstrated in enhancing the foraging and anti-predator behaviour of fish (Laland & Williams 1997; Brown & Laland 2001). For example, in a behavioural experiment with guppies (*Poecilia reticulata*), naïve fish escaped more quickly from a simulated predator in the presence of a pre-trained individual, and overwhelmingly followed that fish rather than utilising an alternative escape route (Brown & Laland 2002). Thus, induced shoaling could promote social learning and enhance behavioural responses to habitat desiccation. Further, it was interesting that only a few small *N. burrowsius* were caught in the Confluence habitat during 1999 considering that the chamber later found below appeared to be frequently utilised, by a large number of fish. If larger *N. burrowsius* were present then they must have already sought refuge in the gravels below. This implies that some *N. burrowsius* actively seek refuge before the habitat becomes totally desiccated.

Use of underground refuge

A distinctive specialisation of *Neochanna* species is a strengthened upper jaw region of the cranium (McDowall 1997a). The increased ossification and proximity of these bones may be an adaptation to burrowing behaviour, such as, head probing. Thus, *N. burrowsius* may be quite capable of forming rudimentary burrows. Indeed, Tasmanian mudfish (*Neochanna cleaveri*) created progressively complex burrows, first vertically and then horizontally, as water was slowly lowered in an aquarium (Koehn & Raadik 1991). Abilities, such as, mouth excavation are not necessarily required to create burrows. Head probing and lateral body oscillations have been documented as methods used by fish to burrow into soft sediment (Atkinson et al. 1987). However, Eldon (1979c) concluded that *N. burrowsius* were only able to widen existing cracks or weaknesses after laboratory investigations revealed little burrowing behaviour in response to emersion. Thus, *N. burrowsius* may require particular sediment characteristics, a pre-existing weakness in the substratum, and/or particular stimuli to initiate burrowing. The consistency of substratum is very important in other burrowing fish, for example snake blenny (*Lumpenus lampretaeformis*), waited six weeks after the substratum had been disturbed before burrowing in a laboratory setting (Atkinson et al. 1987).

The occurrence of *N. burrowsius* in burrows, chambers and cavities has been well-documented (Phillipps 1926; Cadwallader 1975a; Eldon 1979c). Thus, there is little doubt that *N. burrowsius* utilise such refugia during drought. Further, as this behaviour also occurs in *N. apoda* (Eldon 1978). However, individual *N. burrowsius* failed to construct burrows under controlled conditions and at some sites large numbers of fish have been stranded after water levels dropped (Eldon 1979c). Thus, is the use of underground refuge opportunistic or does it represent an actual behavioural response to drought situations? An intentional behavioural strategy would imply that some level of preparation occurs or that *N. burrowsius* habitually use burrows. Eldon (1979c) suggested that *N. burrowsius* habitually burrow into loose vegetation for general cover. Indeed, the stream banks of Hororata Spring contained numerous indentations (Figure 7.6), and similar holes have been found at other sites (Eldon 1979c; personal observation). It is possible that burrow formation takes some time and energy with indentations being aborted attempts at creating a burrow (Eldon 1979c). It is not known what may prompt burrowing behaviour. However, it is likely that during hypoxic conditions *N. burrowsius* may benefit by utilising refuge at the oxygen rich water surface,

potentially explaining the observation of *N. burrowsius* resting among willow roots at the water surface. Furthermore, Cadwallader (1975a) found *N. burrowsius* created burrows at or just above the water level, in a consistently full aquarium. These observations imply that *N. burrowsius* may habitually use such refugia. However, *N. burrowsius* were not found in artificial burrows placed at Hororata Spring when the water level was high and well oxygenated (O'Brien unpublished data).

The discovery of a large cavity containing numerous *N. burrowsius* was serendipitous and such a large congregation of emersed *N. burrowsius* has not been recorded previously. Eldon et al. (1978), and Eldon (1979c) found emersed *N. burrowsius* only singly or in small groups, and in one search only 0.4 % of the minimum estimate of the pre-drought population was found. In the present study, although the cavity was relatively cool and moist due to its depth, substantial mortality was recorded. Furthermore, mortality in emersed *N. burrowsius* was highest in a band of clay substratum. This mortality is likely due to low oxygen availability in clay, whereas, the more porous sandy substratum may permit oxygen diffusion. Thus, the location of burrows is likely to be critical to survival. In my study, mortality due to drought was at least 25 %, although this estimate does not include the large number of inseparable skeletons found. In a thorough study of a Mid Canterbury stream, 30 % mortality of a *N. burrowsius* population occurred during drought conditions (Eldon et al. 1978). Whereas, in laboratory investigations 40 % mortality in *N. burrowsius* occurred after 85 days of emersion (Meredith 1985). Thus, the strategy of emersion is risky and considerable mortality occurs when a habitat dries, even if suitable refuge is available.

Figure 7.6. Holes and indentations formed in stream bank at Hororata Spring. Photo taken during a period of habitat desiccation, usual water level is indicated by vegetation above bare stream bank.

Size-dependant strategies

During emersion, fish must cope with a progressive series of challenges including, movement without water, gas exchange during emersion, toxic waste accumulation, and starvation (Meredith 1985; McPhail 1999). Since surviving such conditions may rely on physiological processes that are affected by size, it is not surprising that fish responses to drought and hypoxia vary according to fish size. Meredith (1985) suggested the presence of an upper and lower limit for surviving drought conditions, stating that the success of *N. burrowsius* in drought prone habitats was due to a strategy of rapidly growing above a minimum size, then remaining within a limited size range. The present study supports this theory and indicates a likely mechanism related to the severity of drought and size-selective mortality. Importantly, there were significant differences in the sizes of fish successfully adopting the differing strategies of immersion and emersion. My results indicate that larger fish may actively leave the water and find refuge, leaving smaller fish still immersed. Smaller fish are likely able to remain immersed as they have lower oxygen demands and higher hypoxia tolerance due to higher surface area to volume ratios. Burleson et al. (2001) in experiments within oxygen gradient chambers found that smaller largemouth bass (*Micropterus salmoides*) had broader hypoxic tolerance levels and utilised water of lower dissolved oxygen more readily than larger

conspecifics. Larger fish, on the other hand, have well developed respiratory systems and are likely to survive emersion for longer periods due to lower metabolic rates. For example, large bluegill (*Lepomis macrochirus*) individuals have greater condition after winter starvation, than smaller con-specifics (Cargnelli & Gross 1997). Indeed, laboratory experiments have confirmed that during emersion, small fish lose weight faster than larger individuals of both *N. burrowsius* and black mudfish (*Neochanna diversus*; Meredith 1985; McPhail 1999). Furthermore, small *N. diversus* showed the greatest reduction in hepatic glycogen, an important energy source, during starvation and aestivation (Davidson 1999).

Both emersion and immersion strategies are risky, however. Although immersed fish can continue to feed, they will also continue to be preyed upon by predators, which can limit the value of pools as drought refugia for small fish (Magoulick & Kobza 2003). Emersed fish however, risk desiccation and starvation. In the present study, emersed and immersed *N. burrowsius* had the same mean relative condition, though the range of condition values varied. Meredith (1985) found that immersed, but starved *N. burrowsius*, lost weight more rapidly than emersed fish. Similar results were obtained in a laboratory study conducted on *N. diversus* (Dean 1995). Thus, remaining active represents a significant energetic cost for immersed fish. Food resources are likely to be scarce and patchy during periods of hypoxia, which may explain the large variation in condition of the immersed fish observed at Hororata Spring. Thus, remaining immersed, as a strategy for survival, will be reliant on the presence of adequate food resources, which is often not the case in remnant pools during drought (Lake 2000).

In summary, the occurrence of facultative shoaling behaviour during drought conditions illustrates the considerable behavioural plasticity of *N. burrowsius*. The ability to use burrows is likely to have important ecological consequences for the persistence of *N. burrowsius* on the increasingly drought-stricken Canterbury Plains. Finally, the investigations detailed in this chapter demonstrate that *N. burrowsius* of differing size do employ varying survival strategies in the field and drought does cause size-selective mortality. Thus, this field-based study supports numerous laboratory studies and physiological theory which have emphasised the importance of size in determining the affects of drought on fish.

Chapter 8.

Competition and co-existence between *Neochanna burrowsius* and *Gobiomorphus breviceps*.

INTRODUCTION

Competition is a simple concept that nonetheless can take multifarious forms. Generally, organisms compete for resources that may increase their fitness via survival, growth, and/or reproductive output. In fish, this may include prey, predator refuge, feeding position, and nest sites (Fausch & White 1981; Mittelbach & Chesson 1987; Glova et al. 1992; McIntosh et al. 1992; Barrier & Hicks 1994; Hamilton 1998). There have also been many approaches to determining the occurrence of inter-specific competition. Patterns of exclusion and resource segregation between species have often been reported as evidence that competition is occurring (e.g. Laughlin & Werner 1980). However, this approach has been largely reproached as mechanisms other than competition can generate similar patterns (Begon et al. 1990). Moreover, such patterns do not prove that interactions between species result in the fitness of one species being reduced, which is integral to the definition of competition. Weber & Fausch (2003) highlighted the need for direct tests of competition and emphasised the distinction between weak and strong evidence for competition, indicating that acquiring strong evidence involved replicated manipulative experiments. However, results obtained in experimental conditions also need to be related to field situations and observations. Thus, to determine that competition between species occurs it is important to examine interactions over several resources and in both field and experimental situations.

New Zealand's indigenous fish are considered to have low competitive ability, due to low community saturation, and the country's geological isolation (McDowall 1968). The focal species of this study, the threatened Canterbury mudfish (*Neochanna burrowsius*), in particular is considered to have sparse experience with competition, and to be a weak competitor (Skrzynski 1968; Cadwallader 1975a; Eldon 1979a; Meredith 1985; McDowall 1990). However, no studies have directly investigated the competitive ability of *N. burrowsius*. The main reasoning supporting the suggestion that *N. burrowsius* is a weak competitor is likely based on the theoretical trade-off between competition and disturbance.

Whereby, species are either strong competitors, or are tolerant of extreme environmental conditions, allowing them to avoid inter-specific interactions (Begon et al. 1990). Certainly, *N. burrowsius* can persist in habitat with extremely low dissolved oxygen levels and thus seldom co-exists with other fish species (Eldon 1979a). Indeed, *N. burrowsius* has been found on its own in 60 % of recorded occurrences of the species (New Zealand Freshwater Fish Database (NZFFD; McDowall & Richardson 1983) records downloaded 16 May 2005). Meredith (1985) also suggested a lack of aggression, low metabolic rate and a small mouth as reasons why *N. burrowsius* would be intolerant of other fish species. However, *N. burrowsius* does often co-occur with the upland bully (*Gobiomorphus breviceps*; 23 % of *N. burrowsius* NZFFD records). *G. breviceps* differs from *N. burrowsius* in having a rigid intra-specific territorial system (Cadwallader 1975b). Further, *G. breviceps* can be aggressive, and has a greater weight to length ratio than *N. burrowsius*, which may provide advantages during interactions (McDowall 1990; Hamilton 1998). This gobioid has the ability to utilise a wide variety of habitats and has a much broader distribution than *N. burrowsius* (Jowett & Richardson 2003). It would be of concern if this common species was limiting the distribution of the threatened *N. burrowsius*. When these two species co-exist it has been observed that *N. burrowsius* occurs in aquatic macrophyte beds, whereas *G. breviceps* occupies stony pool areas (Eldon 1979a). Thus, patterns of habitat segregation, suggestive of negative inter-specific interactions are evident.

The magnitude and nature of competition between species will depend, in part, on how similar their resource requirements and behaviours are (Begon et al. 1990). The diets of New Zealand's fish fauna typically show substantial overlap (McDowall 1990). Thus, competition for food among native fish is likely to be common, especially during periods of limited food resources, such as summer low flows (Sagar & Glova 1994), and after floods (Scrimgeour & Winterbourn 1987). Analysis of the diets of *N. burrowsius* (Eldon 1979b), and *G. breviceps* (Cadwallader 1975c; Staples 1975), indicate that both are generalist opportunistic carnivores, feeding on any slow moving aquatic invertebrates of an appropriate size encountered. These species are temporally segregated with *G. breviceps* being diurnal (Sagar & Glova 1994), whereas *N. burrowsius* forages nocturnally, probably being able to detect prey non-visually by olfactory sense and accessory lateral lines (Eldon 1979b, McDowall 1997b). The importance of temporal segregation as a mechanism of co-existence in the New Zealand fish fauna has been noted, with Sagar & Glova (1994) hypothesising that species foraging during different photo-periods are more likely to have similar dietary compositions.

Competition for resources is often greatest when shared resources are limited (Begon et al. 1990). As with many threatened species, *N. burrowsius*' habitat has been removed, in this case due to the transformation of wetlands into agricultural land (Skrzynski 1968; Cadwallader 1975a; Eldon et al. 1978; Eldon 1979a; McDowall 1998). Waterway channelisation has also rendered remaining habitat more suitable to other fish species, which may compete with *N. burrowsius* (Eldon 1979a). Moreover, modern agricultural management practices impart regular disturbances on waterways, generating a homogenous habitat by removing aquatic vegetation and habitat structure (Hudson & Harding 2004). This is likely to reduce the amount of refuge available, intensifying inter-specific competition for remaining refuge, the lack of which may increase predation risk (Portt et al. 1986; Mittelbach & Chesson 1987). Although freshwater eels (*Anguilla* spp.) are opportunistically piscivorous, New Zealand contains few specialised piscivorous fish; thus bird species are often the dominant predators of fish (McDowall 1968). Both *N. burrowsius* and *G. breviceps* grow to less than 160 mm in length, making them vulnerable to avian predators such as white faced herons (*Ardea novaehollandiae*; Readers Digest 1985). As avian predators forage visually, cover is likely to be important in avoiding predation for small fish. Thus, the outcome of inter-specific competition between *N. burrowsius* and *G. breviceps* over refuge may have consequences for survival.

In this chapter I detail a series of investigations carried out to determine if the presence of *G. breviceps* is likely to disadvantage *N. burrowsius* populations. To answer this question I conducted field studies into the habitat use and diet of *N. burrowsius* and *G. breviceps* in sympatry, and tank investigations into interactions over refuge, using both additive and substitutive experimental designs. These two experimental designs examine different questions, whereby additive designs determine the occurrence of competitive interactions and substitutive experimental designs determine the magnitude of inter- versus intra-specific competition (Weber & Fausch 2003). Overall, it was hypothesised that *G. breviceps* would have a competitive advantage when interacting over spatial and prey resources, and that this would have a significant adverse effect on the refuge use and growth of *N. burrowsius*.

METHODS

Field investigations

Habitat

Habitat segregation between *N. burrowsius* and *G. breviceps* was examined in the field. Preliminary sampling indicated that *N. burrowsius* and *G. breviceps* populations co-existed in two of the four main study sites, i.e. Te Roto Repo o Tawera and Hororata Spring. Within these two sites, baited Gee minnow traps (GMT; 2 x 2 mm mesh) were placed either in macrophyte patches or open areas using a stratified random design. To test whether there were differences in the spatial occurrence between species the abundance of each species captured in each GMT was compared using Spearman rank correlation and ANOVA. Gee minnow traps that did not capture fish were not included in the analysis.

Diet

During the summer of 1999, Gee minnow trapping was conducted at Hororata Spring. Unfortunately, low dissolved oxygen levels resulted in the deaths of both *N. burrowsius* and *G. breviceps* juveniles within traps placed in the Willow Pool (see Chapter 1 for habitat description). During sampling 40 % of *G. breviceps* captured in traps died, compared with only 11 % of *N. burrowsius*. Dead fish retained from this incident were used to investigate differences in diet composition of *N. burrowsius* populations in sympatry with *G. breviceps*. Stomach contents were examined from twelve *G. breviceps* and four *N. burrowsius*. Although this sample size is small it was still worth examining, as Eldon (1979b) compared the results of a large and small study involving 367 and 32 *N. burrowsius* and reasoned that sample size did not alter conclusions. All fish were < 70 mm total length (TL). Mean length (± 1 SE) was 51 ± 3.1 mm TL for *G. breviceps*, and 47 ± 1.5 mm TL for *N. burrowsius*. Dead fish were transported to the laboratory in 70% ethanol, and then stored in 5% formalin until further processing. Stomach fullness was estimated using a method similar to Eldon (1979b), where 40 points indicated a distended stomach, 32 an apparently full stomach; 16 a half full; and 8 points an estimated quarter full stomach. Stomachs were then dissected out and the contents mounted on glass slides with Lactophenol-PVA containing Rose Bengal dye. Individual invertebrates were identified and counted under magnification, using recognisable features. Where possible Cladocera were identified to genus using the keys of Chapman & Lewis (1976), with abundance estimated by post-abdominal claw counts. Three genera of Cladocera were identified, most commonly the planktonic *Ceriodaphnia* as well as the less

active *Simochephalus* and *Sayciinae* (Chapman & Lewis 1976). Cyclopoid copepods were commonly found in pieces, thus their abundance was estimated by counting combined body sections. Pairs of ostracod valves were used to count one whole individual. Chironomidae were identified and counted by their menta plates using the keys of Winterbourn et al. (2000). *Culex* sp. were counted by their siphons. Terrestrial organisms were grouped together and counted by their head capsules and/or menta plates. Other organisms were identified whole, while unidentifiable fragments were not used in the analysis. Forward stepwise discriminant analysis was used to identify prey taxa that differentiated between the diets of *N. burrowsius* and *G. breviceps*.

Experimental investigations

To determine whether patterns of habitat segregation observed in the field were due to competition, experiments were conducted in twelve 750 litre, modified cattle troughs situated outdoors. Maximum tank diameter was 1.54 m across the top, area of the tank floor was 1.85 m^2, and depth was 0.4 ± 0.1 m (mean ± 1 SE). Water levels were controlled by ball cocks and excess rainwater escaped through overflow holes (5 mm diameter). Minimum water level and continuous outflow were maintained by a 5 mm diameter hole set at 10 mm above the tank bottom. Water flow through each tank was approximately 1 litre/min, sourced from an aquifer bore. Tanks were seeded with invertebrates obtained from a fishless pool on the Selwyn River flood plain and allowed to colonise for one week before experiments commenced. These invertebrates were predominately micro-crustaceans, *Culex* sp. and Chironomidae, and were present in tanks throughout the period of the experiments.

Competition for refuge

Three experiments were conducted to assess temporal and spatial patterns in refuge use by *N. burrowsius* in the presence and absence of *G. breviceps*. The first two experiments assessed the refuge use of *N. burrowsius* only; during the day, when fish would potentially be seeking refuge; and during the night, when they would likely be foraging. The third investigation assessed refuge use of *N. burrowsius* and *G. breviceps*, in the presence of each other, during the day using an additive experimental design.

N. burrowsius used for this experiment had no previous experience of *G. breviceps* as they were sourced from a long-term captive population in Lake Eldon; see description by Eldon (1986). *G. breviceps* were sourced from the Okuku River and had no previous experience of

N. burrowsius. *N. burrowsius* were 71.6 ± 1.1 mm TL (mean total length ± 1 SE), and 1.5 ± 0.06 g (mean ± 1 SE); whereas *G. breviceps* were 69.2 ± 1.7 mm TL (mean total length ± 1 SE), and 3.7 ± 0.35 g (mean ± 1 SE).

Experimental substratum consisted of the three common substrata types found in the natural habitats of *N. burrowsius*, i.e. cobbles, leaf debris, and fine sand. Stone substratum consisted of greywacke cobbles collected from the Selwyn River. Debris substratum composed of leaves, twigs, and detritus; collected from Selwyn River margins and weighed down in tanks by several 20 cm lengths of 4 mm diameter wire. Sand substratum was fine river sand (0.125 – 0.5 mm) commercially collected and graded, from the Waimakariri River. Two substrata types were placed in each tank. Thus, three experimental treatments were formed by pair-wise permutations of the three habitat patches, with the use of 12 tanks allowing four replicates per treatment. Each substratum type covered nearly half of the tank floor. Treatment pairs and the position of each substratum type were randomly assigned among tanks.

Refuge use by *N. burrowsius* was sampled in the absence and subsequently in the presence of *G. breviceps*. This temporal segregation allowed greater replication, and environmental conditions did not change substantially during the experiment. Ten *N. burrowsius* from the captive population were randomly assigned to each tank. They were acclimated for one week, before being sampled during the day (2 November 1999), and night (4 November 1999). Ten *G. breviceps* were then introduced to each tank, acclimated for one week, with substratum utilisation of both species being sampled during the day (9 November 1999).

Sampling was conducted by placing a mesh separator across the middle of the tank. Sand filled fabric 'sausages' (0.06 m diameter, 1m long) were then used to seal the sides and bottom of the separator to prevent fish movement between the two halves of the tank during sampling (Figure 8.1). Separation of tanks for both day samples was conducted between 10:30 and 11:30 (New Zealand Daylight savings Time, NZDT). Night sampling was conducted between 20:00 and 20:30 (NZDT). Once the separators had been placed fish were captured in each tank, with the number in each substratum type being recorded.

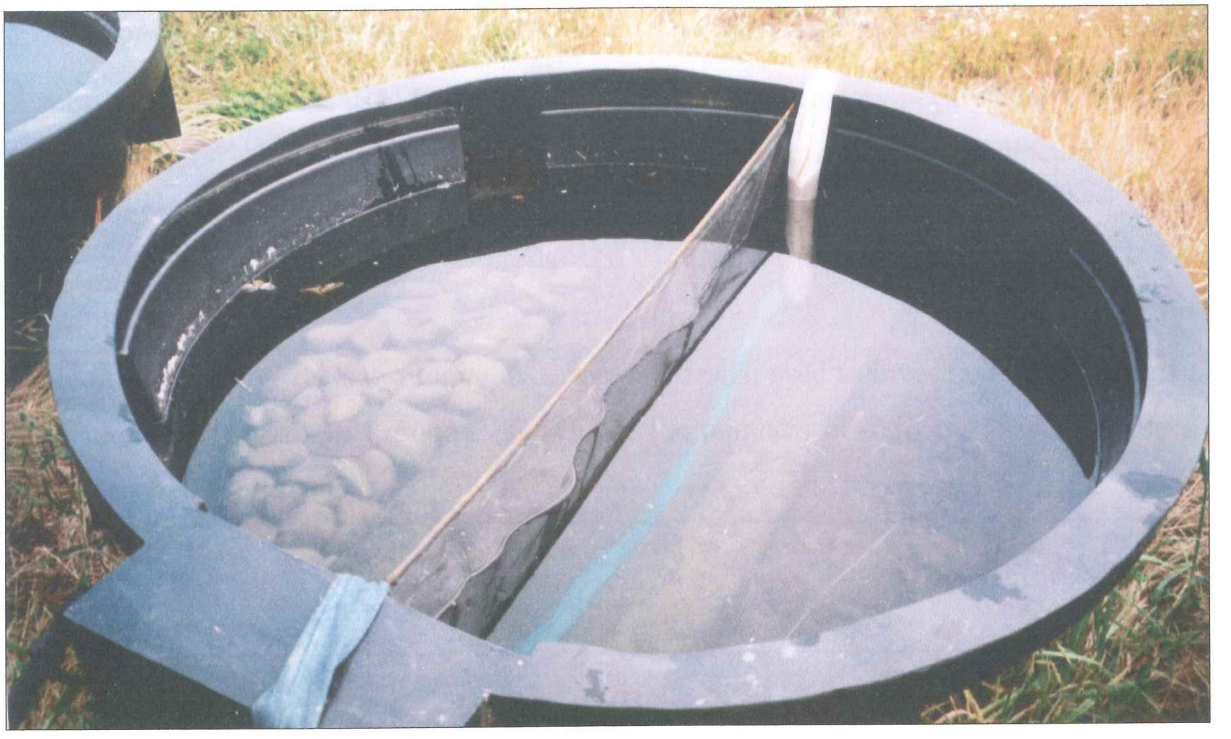

Figure 8.1. Modified cattle trough used as experimental tank with mesh separator in place.

As total fish density in tanks was known, the number of fish in one substrata type was the inverse of its paired substrata type. To enable analysis of treatments, the three substrata were ranked stone > debris > sand, according to the assumed level of refuge they provided. Stone was ranked best as it provides a complex static refuge, with complete cover. Debris can also provide complete cover, however it is easily moved. *A. novaehollandiae* often 'foot rake' during feeding, moving substrate and startling fish into the open (Readers Digest 1985), thus debris were ranked as providing less refuge than stone substratum. Sand was ranked lowest, as it provides little cover.

The level of preference between substrata types was analysed by testing the significance between proportions using the z-test statistic. Comparisons between trials were analysed using contingency tables and Chi-square tests on the number of *N. burrowsius* in each refuge type.

Effect of G. breviceps *on* N. burrowsius *growth*

A substitutive design was used to investigate the magnitude of inter-specific competition from *G. breviceps* on *N. burrowsius* growth, compared with intra-specific competition for *N. burrowsius* alone (Table 8.1).

Table 8.1. Details of the substitutive experimental design used to investigate the affect of *G. breviceps* on *N. burrowsius* growth. Given are the numbers of individuals of each species used in each of four treatments.

	Low density		High density	
N. burrowsius	6	3	12	6
G. breviceps	0	3	0	6

Growth rates of individually marked *N. burrowsius* were measured in the presence and absence of *G. breviceps* over a 31 day period from 3 March – 3 April 2000. The previous experiments indicated that competitive interactions between *N. burrowsius* and *G. breviceps* may be most intense in cobble substratum. Thus, in this experiment the tank substratum consisted entirely of cobbles. Supplementary feeding of approximately 8 g frozen chironomids per tank per day was given, in addition to invertebrate prey being present in the tanks throughout the experiment. Minimum/maximum thermometers placed in each tank recorded a maximum temperature of 27.8 °C ± 0.94 (mean ± 1 SE) and a minimum temperature of 8 °C ± 0.28 (mean ± 1 SE) over the duration of the experiment.

Change in individual fish condition was calculated as the difference in relative condition of *N. burrowsius* between the beginning and end of the experiment. Relative condition was calculated from power regressions on fish length – weight data for both measurement dates using the equation $Kn = W / a \cdot L^b$, where Kn is relative condition, W is weight (g), and L is length (mm), a is the intercept and b is the slope of the regressed power equation. Refer to Chapter 5 for further description of relative condition. *N. burrowsius* have differential growth (Eldon 1979c). Thus, in this experiment larger fish gained less weight and length than smaller fish (weight gain (g/31 days) = -0.19·Initial weight (g) + 0.9, $R^2 = 0.36$; growth in length

(mm/31 days) = -0.14·Initial length (mm) + 15.8, R^2 = 0.42). Thus, initial size was included as a covariate in statistical analyses. Growth of *N. burrowsius* was analysed using analysis of co-variance (ANCOVA), with variables being log transformed where necessary.

Differences in trapability

At the completion of the growth experiment, Gee minnow traps were used to recapture the fish from the tanks. This provided the opportunity to investigate differences in trapability, which may suggest differences in activity levels and foraging behaviour of *N. burrowsius* in the presence and absence of *G. breviceps*. Data were analysed using the median test, which frames the computation in terms of a contingency table, and is particularly useful when the scale contains artificial limits with many cases falling at either extreme of the scale, as was apparent in my data (StatSoft Inc. 2001).

RESULTS

Field investigations

Habitat use

During field studies, small scale differences in habitat use were identified, supporting Eldon's (1979a) observations of habitat segregation between *N. burrowsius* and *G. breviceps*. Analyses of trap survey data indicated a negative relationship between the number of *N. burrowsius* and the number of *G. breviceps* captured in each Gee minnow trap (Spearman R = -0.24, p = 0.03, n = 81; Figure 8.2). In particular, traps that caught large numbers of *G. breviceps* caught few *N. burrowsius*. Furthermore, *N. burrowsius* appeared to utilise macrophyte patches more frequently than open areas, whereas *G. breviceps* were captured more often in open stony areas (Figure 8.3). This is supported by the results of factorial ANOVA, which indicated a significant interaction between the abundance of each species, in each patch type ($F_{(1, 102)}$ = 4.8, p = 0.03; Figure 8.3).

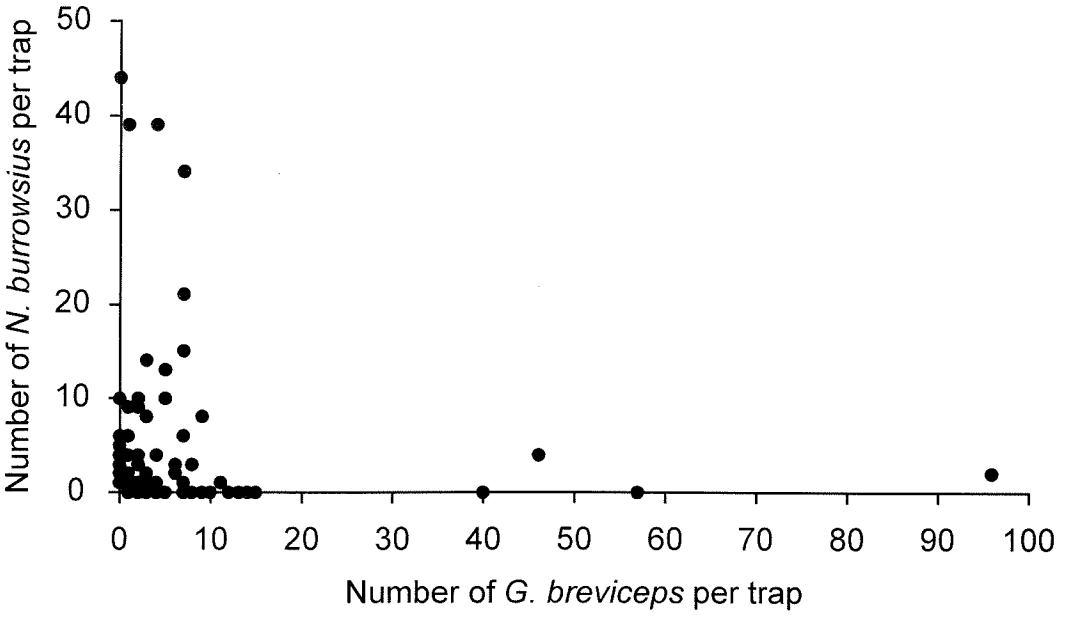

Figure 8.2. Relationship between numbers of *N. burrowsius* and *G. breviceps* in individual Gee minnow traps from sampling conducted at Te Roto Repo o Tawera and Hororata Spring during autumn 1999 and 2002. Data from traps that did not catch fish were not included.

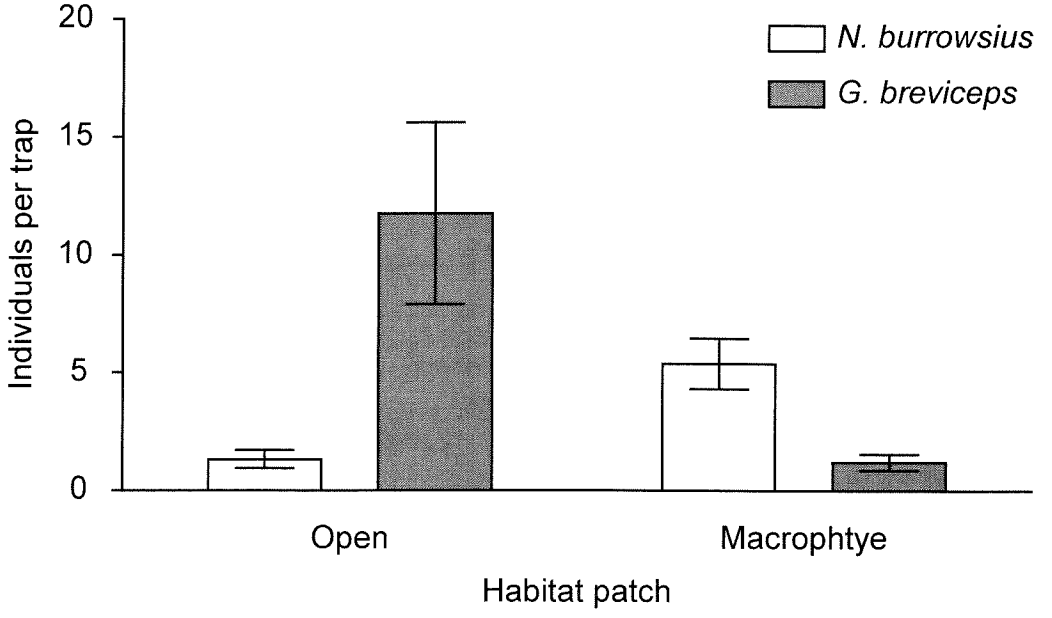

Figure 8.3. Abundance (mean ± 1 SE) of *N. burrowsius* and *G. breviceps* captured in individual Gee minnow traps placed in macrophyte patches and in open areas at Te Roto Repo o Tawera and Hororata Spring during sampling in 1999.

Diet

Stomach content analysis indicated that the diet of juvenile *N. burrowsius* was comprised of micro-crustaceans and dipterans, mainly. At Hororata Spring, both *G. breviceps* and *N. burrowsius* juveniles fed predominantly on crustaceans, particularly copepods (Table 8.2). However, *G. breviceps* consumed, on average, twice as many prey items compared to *N. burrowsius* juveniles. Furthermore, *G. breviceps* consumed a greater range of prey items, and exhibited greater stomach fullness (Table 8.2).

Forward step-wise discriminate analysis differentiated the diet of juvenile *N. burrowsius* from that of *G. breviceps* ($F_{(3, 15)} = 8.1$, $p = 0.002$, Eigenvalue = 1.6). Of the eleven invertebrate taxa initially included as variables in the discriminate model, three taxa differentiated between the diets of the two species. These were terrestrial invertebrates, copepods and ostracods, having canonical coefficients of 0.68, -0.7, and -0.64, respectively. The discriminant model, based on numbers of invertebrate individuals per fish, correctly classified 88 % of *N. burrowsius*, and 91 % of *G. breviceps* samples. The predominant difference between the species was that *N. burrowsius* juveniles consumed terrestrial invertebrates, whereas *G. breviceps* consumed ostracods and a greater number of copepods (Table 8.2).

Table 8.2. Diet composition of juvenile *N. burrowsius* and *G. breviceps* at Hororata Spring. Mean values are given for visually assessed stomach fullness on a scale of 0 – 40 points (see text for more details), total mean number of prey items per juvenile and the mean number of prey per fish in each invertebrate taxa group.

	G. breviceps	*N. burrowsius*
Sample size	12	4
Stomach fullness score	24	16
Prey items per fish	20	12
Taxa group	Mean number per fish	
Crustacea		
Cladocera	3.1	1.5
Copepoda	14.3	7.3
Ostracoda	0.5	
Diptera		
Chironomus zealandicus	0.9	0.3
Other		
Oxythira sp.	0.1	
Physa sp.	0.1	
Rotifer	0.2	
Oligochaeta	0.2	
Acarina	0.2	
Terrestrial invertebrates		1.5
Unidentified eggs	0.1	1

Experimental investigations

Competition for refuge

Refuge use by *N. burrowsius* in the absence of *G. breviceps* was similar during the day and night (Figure 8.4a and b; $\chi^2 = 4.1$, df = 2, p = 0.1). *N. burrowsius* utilised stones and debris refuge in preference to sand, which provided little refuge. However, there was less difference in the numbers of *N. burrowsius* found in stone patches compared with debris, with debris being utilised to a greater extent (Figure 8.4a and b). With the addition of *G. breviceps*, refuge choice by *N. burrowsius* changed significantly (Figure 8.4a and c; $\chi^2 = 22.4$, df = 2, p < 0.0001). Further, there was a significant difference between the refuge use of each species when occurring together (Figure 8.4c and d; $\chi^2 = 22.4$, df = 2, p < 0.0001). *G. breviceps* exhibited similar patterns of refuge use as *N. burrowsius* did when alone, although the level of preference was not as strong as indicated by z-test comparisons (Figure 8.4d). The displacement of *N. burrowsius* by *G. breviceps* was most pronounced in treatments containing stone substratum (Figure 8.4c). Most importantly, in the presence of *G. breviceps*, *N. burrowsius* sought refuge in sand patches that provided little cover, rather than remain in stone substratum. When the treatment choice did not include stone substratum *N. burrowsius* maintained their use of debris in preference to sand (Figure 8.4c). Thus, interactions with *G. breviceps* that resulted in a change in refuge use by *N. burrowsius* were associated with the presence of stone substratum. In summary, the presence of *G. breviceps* resulted in displacement of *N. burrowsius* from stone substratum, leading to greater utilisation of debris and little preference in refuge use between stone and sand patches, despite these substratum types providing differing levels of cover (Figure 8.5). Furthermore, the presence of debris and no stones in a tank appeared to moderate interactions over refuge as displacement was not evident (Figure 8.4b).

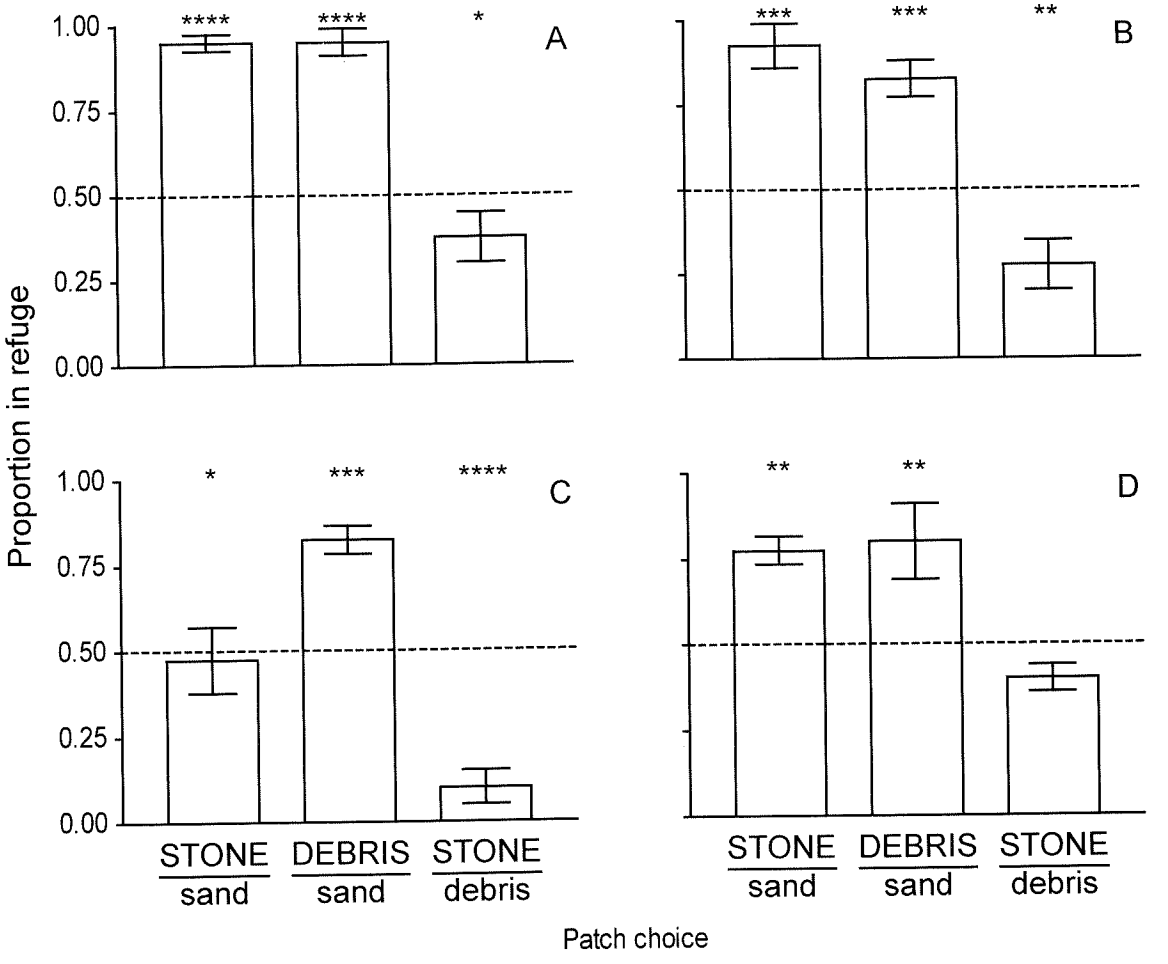

Figure 8.4. Mean (± 1 SE) proportion of fish in substrata assumed to provide refuge of higher quality (upper case) over the alternative choice (lower case) for each pair-wise treatment. Graphs show refuge use of (A), *N. burrowsius* during the day, (B), during night, and (C), in the presence of *G. breviceps*, as well as (D), refuge use of *G. breviceps*. Dotted lines indicate the result expected if individuals were evenly distributed between substrata types. Asterisks indicate the number of replicates in which fish showed significant levels of substratum preference as determined by z-test statistics.

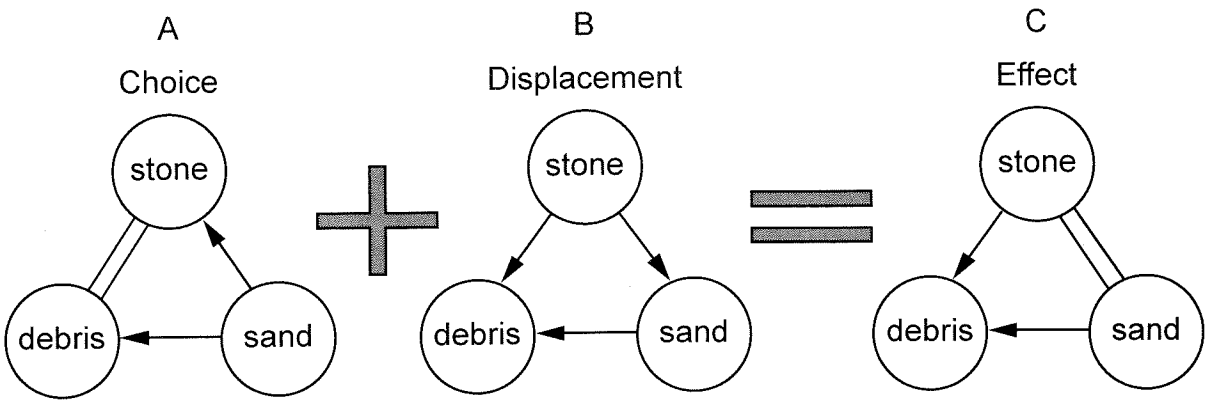

Figure 8.5. General summary of the interactions between *N. burrowsius* and *G. breviceps* in relation to substrata type. When *N. burrowsius* occurred on its own (A), it tended to use both stone and debris patches equally and avoided sand. The presence of *G. breviceps* displaced *N. burrowsius* from stone patches, but when stone refuge was absent, *N. burrowsius* still avoided sand (B). This displacement resulted in (C), the observed distribution of *N. burrowsius* in the presence of *G. breviceps*. Circles represent each of the substrate choices in the experiment; stone, sand, and debris. Between these circles, arrows indicate habitat preference, whereas, double lines indicate that there was little difference in the proportion of *N. burrowsius* within substrates.

Spawning in G. breviceps

A serendipitous result of the above experiment was that *G. breviceps* spawned in tanks. Fish were maintained in the tanks, as per the experimental set-up for several months after the conclusion of the experiment. Recently hatched *G. breviceps* fry were found on 28 December 1999 in three of the four tanks containing stone and debris substratum patches. No fry were ever found in tanks with sand and debris, or sand and stone combinations, suggesting that either spawning was inhibited, as these conditions were not suitable for *G. breviceps*, or high rates of cannibalism occurred in these tanks.

Effect of G. breviceps *on* N. burrowsius *growth*

Substitutive experiments investigating competitive interactions over stone substratum indicated that in general, total fish density had a greater affect on *N. burrowsius* growth than the presence of *G. breviceps* (Table 8.3). Thus, there was little difference between the effects of intra- and inter-specific competition, especially at high densities (Figure 8.6). However, factorial ANCOVA on the change in length of *N. burrowsius* resulted in a significant interaction term (Table 8.3). At low densities, despite there being no difference in mass

accumulated by *N. burrowsius* in the presence or absence of *G. breviceps*, there was a significant difference in the growth in length and a difference in whether *N. burrowsius* lost or gained condition on average (Figure 8.6). Thus, subtle differences in the way acquired resources were allocated were apparent in low density treatments, but not at higher densities. At low densities, allocation to growth in length was reduced in the presence of *G. breviceps*, resulting in *N. burrowsius* being shorter, yet maintaining greater condition (Figure 8.6). In contrast, when only intra-specific interactions occurred, *N. burrowsius* grew in length, but at the cost of reduced condition (Figure 8.6). The relative condition of *G. breviceps* after the experiment was not significantly different between treatments, due to high variability in the condition of individuals within tanks, for example relative condition in one tank ranged from 0.81 to 1.35.

At the end of the experimental period baited Gee minnow traps were placed overnight in tanks to capture fish for final measurement. Gee minnow traps placed in tanks containing *G. breviceps* captured significantly more *N. burrowsius*, irrespective of density (Figure 8.7; median test $\chi^2 = 5.3$, df = 1, p = 0.02). Thus, *N. burrowsius* were likely more active in the presence of *G. breviceps*, which may indicate that *N. burrowsius* is required to increase foraging activity to compensate for competitive interactions with *G. breviceps*.

Table 8.3. Factorial ANCOVA results examining the change in length, weight and relative condition of *N. burrowsius* at high and low densities (Density) in the presence and absence of *G. breviceps*. Initial length and weight were included as covariates in analyses.

	Length		Weight		Condition	
	$F_{(1,8)}$	*p*-value	$F_{(1,8)}$	*p*-value	$F_{(1,8)}$	*p*-value
Density	8.7	0.018	12.15	0.008	6.50	0.034
G. breviceps	0.7	0.443	0.0	0.976	0.54	0.483
Density x *G. breviceps*	9.2	0.016	0.64	0.446	0.84	0.385

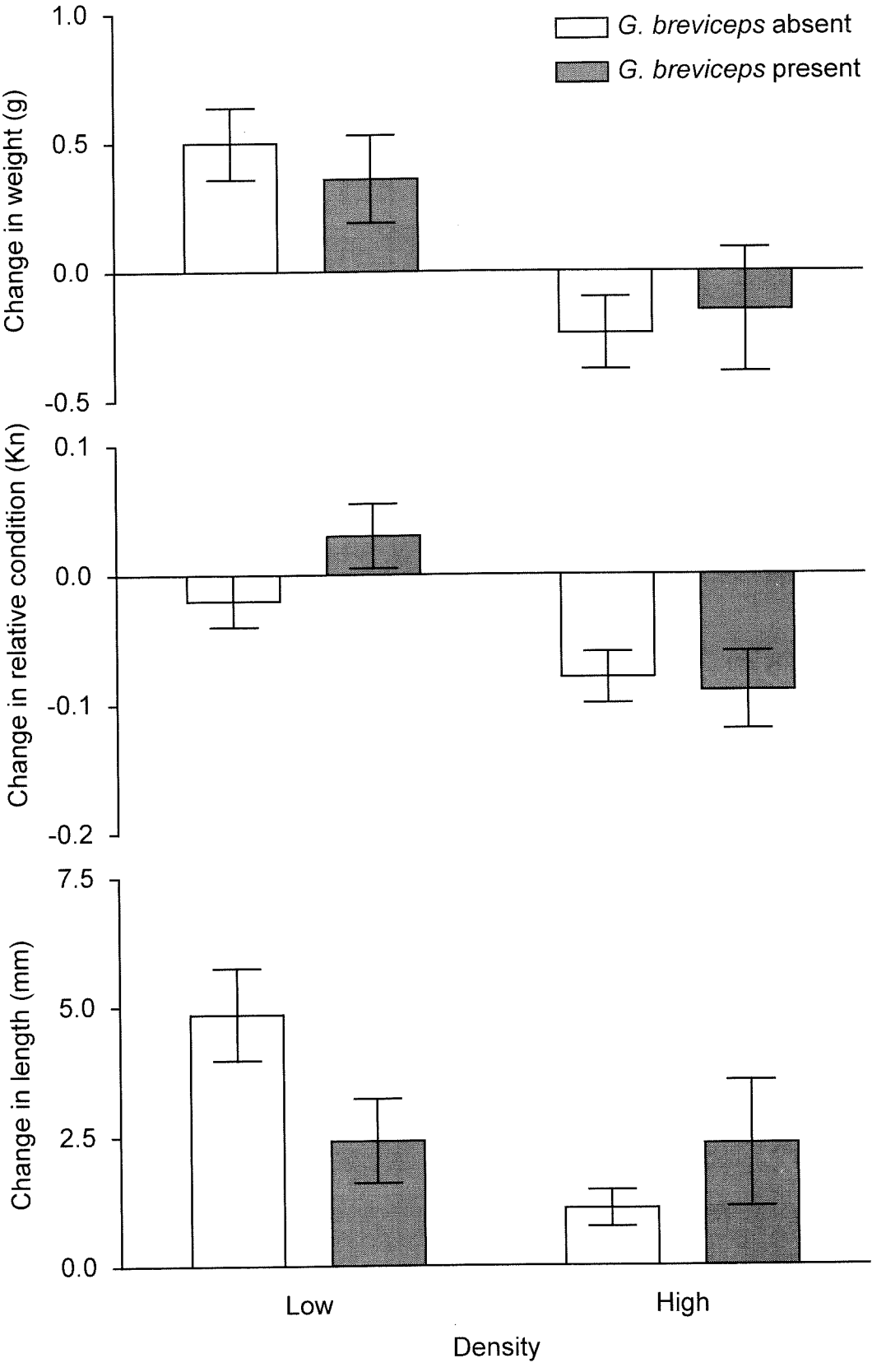

Figure 8.6. Growth of *N. burrowsius* at low (6 fish per tank), and high (12 fish per tank) densities, in the presence and absence of *G. breviceps*. Changes (mean ± 1 SE) in *N. burrowsius* weight, relative condition (Kn), and length, are given over the 31 day period of the experiment.

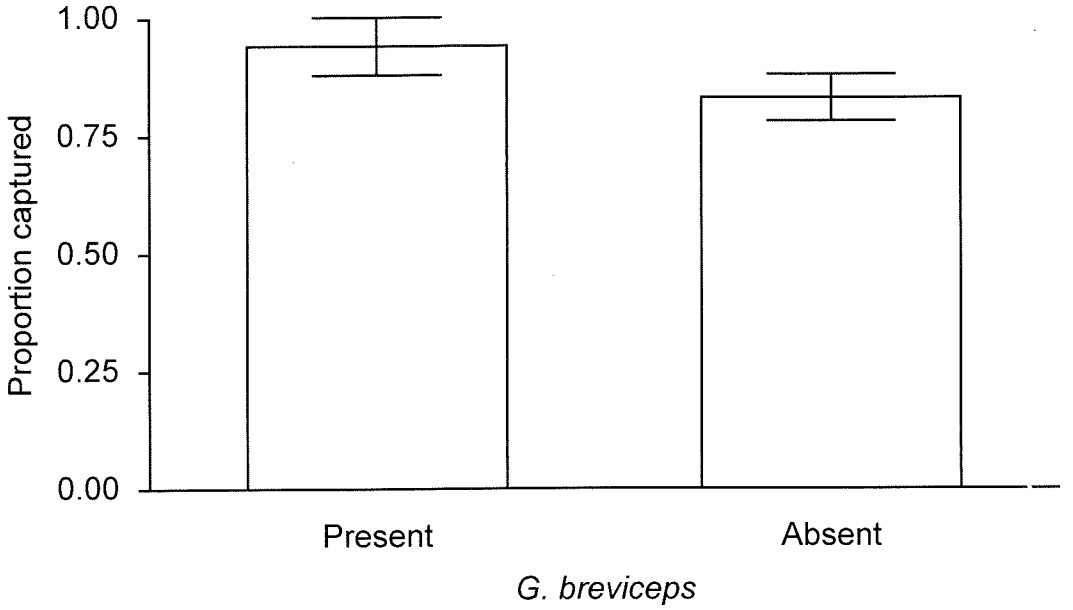

Figure 8.7. Proportion of *N. burrowsius* present captured in baited Gee minnow traps in the presence and absence of *G. breviceps* (mean ± 1 SE).

DISCUSSION

Field studies into the habitat use and prey consumption of *N. burrowsius* and *G. breviceps* indicated that segregation of resources occurs when these species co-exist. Furthermore, the results of experimental investigations indicate that competitive interactions likely contribute to these patterns. However, I found that *N. burrowsius* may not be as adversely affected by the presence of *G. breviceps* as initially supposed.

Competition for food resources

The diets of both *N. burrowsius* and *G. breviceps* juveniles at Hororata Spring were dominated by copepods. This dietary overlap is consistent with that expected for temporally segregated generalists (Sagar & Glova 1994). Copepods are tolerant of hypoxic conditions (Chapman & Lewis 1976), potentially explaining their abundance in stomach contents, and likely the habitat. Indeed, low dissolved oxygen levels contributed to the trapping mortality that provided the opportunity to compare the diets of these species. However, differences between the diets of juvenile fish were also apparent. Juvenile *N. burrowsius* consumed a greater number of terrestrial items, which are usually encountered at the water surface. Whereas, the ostracods consumed by *G. breviceps* are predominately benthic (Chapman & Lewis 1976). Thus, differences in the prey items consumed by juveniles is likely a

consequence of differing foraging behaviours, whereby juvenile *N. burrowsius* are pelagic (Eldon 1979c), whereas *G. breviceps* are typically more benthic (McDowall 1990). However, in addition to differences in the type of prey consumed, *G. breviceps* consumed a greater variety, and quantity of prey items. Eldon (1979b, p. 259) commented that 'slowness was a characteristic of [Canterbury] mudfish feeding habits. They were certainly not sluggish, but gave an impression of leisureliness which is difficult to describe without being anthropomorphic.' Such foraging traits may disadvantage *N. burrowsius* in direct competitive interactions for prey items. Thus, *G. breviceps* is likely to consume a greater proportion of prey resources present. However, this may not necessarily disadvantage *N. burrowsius* as its slow movements are due to its low basal metabolic rate, meaning that its energy requirements are low (Meredith 1985). Thus, *N. burrowsius* may withstand food limitation and starvation for longer periods than *G. breviceps*. Furthermore, the tendency for *N. burrowsius* to consume terrestrially sourced prey items may allow it to supplement its diet if aquatic prey were scarce.

Competition for spatial resources

My results confirm field observations by Eldon (1979a) that when co-occurring *N. burrowsius* is more likely to be found in macrophyte patches and *G. breviceps* in open stony areas. Experimental investigations indicate that this pattern of habitat segregation is likely to be due to the displacement of *N. burrowsius* by *G. breviceps* from areas of cobble substratum. Species may invest more energy in protecting and maintaining resources that are particularly important to them. The displacement of *N. burrowsius* by *G. breviceps* from cobble refuge, but not as strongly from debris, may be because of the value of cobble substratum to *G. breviceps* as spawning substrate, as well as refuge (Hamilton 1998). *G. breviceps* only spawned in some of the experimental tanks and only when stone substratum was present, suggesting that spawning in *G. breviceps* is limited if suitable substratum is absent. Furthermore, *G. breviceps* abundance has been shown to be adversely affected by sedimentation and lack of suitable cobbles both in the field (Jowett & Richardson 1995), and experimentally (Jowett & Boustead 2001). Thus, competition for spatial resources may be limited to cobble substratum, meaning the affect of *G. breviceps* on *N. burrowsius* will be dependent on the characteristics of the habitat that they share.

Optimal foraging theories assume that animals can combine dissimilar factors into a common currency when making choices (Kotler 1984). In my study, the potential cost of predation and

the immediate cost of competition appeared to be perceived as similar. For example, the apparent cost of predation was seen in the avoidance of sand when *N. burrowsius* occurred on its own. However, when *G. breviceps* were present, the opposing cost of interacting with *G. breviceps* within stone substratum resulted in a proportion of fish prepared to risk areas with little cover to avoid competitive interactions. Competition that results in displacement from quality refuge of weaker competitors can result in their increased vulnerability to predation (Söderbäck 1994). For *N. burrowsius*, this may be especially important in situations with low habitat heterogeneity, and thus limited refuge availability, as occurs after mechanical drain maintenance (Hudson & Harding 2004). Indeed, *A. novaehollandiae* have been observed to take advantage of such occasions, being more abundant at a location containing *N. burrowsius* following removal of macrophytes from drains (M. & H. Redshaw, previously Lyall Rd, St Andrews, personal communication). Modern agricultural practices, by removing aquatic vegetation may indirectly limit *N. burrowsius* persistence in waterways containing *G. breviceps* via displacement of *N. burrowsius* into areas where they are more at risk from avian predation. However, displacement does not strictly demonstrate that deleterious competition is occurring (Weber & Fausch 2003). That is, by avoiding competitive interactions with *G. breviceps*, *N. burrowsius* may not necessarily incur any reduction in fitness. Similarly, Werner & Hall (1977, 1979) showed that if bluegills (*Lepomis macrochirus*) and pumpkinseeds (*L. gibbosus*) were allowed to segregate by habitat, competition between them was greatly reduced. Additionally, if sufficient alternative cover is available, displacement may not increase predation risk.

Cost of competitive interactions

There was no difference in the weight gained by *N. burrowsius* in the presence or absence of *G. breviceps* despite experimental tanks having stone substratum. This indicates that *N. burrowsius* acquired similar energy resources in both intra- and inter-specific situations during the one month of experimental manipulations. Thus, competition for spatial resources with *G. breviceps* does not necessarily influence the competitive outcome for *N. burrowsius* in relation to food resources. A similar result was found during investigation of the interactions between *G. breviceps* and the morphologically similar common bully (*Gobiomorphus cotidianus*). *G. breviceps* was able to spatially dominate artificial channels, yet growth and condition of *G. cotidianus* were not negatively affected (Glova 1999). In Glova's study, *G. breviceps* growth and condition were reduced most strongly by intra-specific competition. This may have also occurred in my study, as post-experimental

condition of *G. breviceps* was highly variable within tanks, and was independent of density. This suggests that some *G. breviceps* individuals acquired much greater energy resources than conspecifics. The relative strength of intra- versus inter-specific competition is considered to determine the likelihood of co-existence between species (Begon et al. 1990). Thus, the strength of intra-specific competition in *G. breviceps* may limit their inter-specific interactions and promote co-existence with other species. Furthermore, the ability of *G. breviceps* to compete for spatial resources may be limited in some circumstances, as Cadwallader (1975b) found that *G. breviceps* was displaced by Canterbury galaxias (*Galaxias vulgaris*).

Although *N. burrowsius* weight gain was not significantly different, growth in length was suppressed in the presence of *G. breviceps* at low densities. Furthermore, *N. burrowsius* maintained higher condition, suggestive of higher short term energy storage. This may indicate that qualitative competitive asymmetry occurs whereby competitive interactions result in differing responses in growth attributes, as Resetarits (1997) found between mottled sculpin (*Cottus bairdi*) and fantail darter (*Etheostoma flabellare*). Thus, *N. burrowsius* appear to exhibit plasticity in their energy allocation, possibly allowing *N. burrowsius* to compensate for the presence of *G. breviceps*. The response of maintaining condition at the cost of growth in length may indicate that immediate energy expenditure due to inter-specific interactions is high. Fish can accumulate functional protein for storage and subsequent rapid mobilization (Mommsen 2001). Unlike allocation to muscle protein or lipids, growth in length is a largely irreversible allocation. However, because fecundity in *N. burrowsius* increases nearly exponentially with length (Eldon 1979c) increases in length will have long-term benefits for fitness. Thus, the presence of *G. breviceps* may lower potential fecundity of a *N. burrowsius* population, even when similar resources are acquired, by affecting how energy is allocated to growth. Yet, this response was density dependent. Generally, competition is more intense when resources are limited (Scrimgeour & Winterbourn 1987; Persson & Greenberg 1990; Sagar & Glova 1994). However, *N. burrowsius* showed a more pronounced response to the presence of *G. breviceps* at low fish densities. In high density treatments, food resources were likely limited during the experiment as *N. burrowsius* lost both weight and condition. This likely contributed to the observed result, which may indicate that there is a threshold below which *N. burrowsius* is unable to alter its allocation of energy resources to compensate for competition because food consumption is insufficient to cover metabolic requirements.

Conclusion

There are various mechanisms promoting co-existence between *N. burrowsius* and *G. breviceps*, including temporal segregation, differing diet composition, and the high compensatory ability of *N. burrowsius*. Similarly, Barrier & Hicks (1994) showed that the black mudfish (*Neochanna diversus*) is capable of co-existing with the aggressive Gambusia (*Gambusia affinis*), via differences in feeding activity, life history and environmental tolerances. However, my study also highlights that even when direct energetic costs of competition appear low, adverse consequences of biotic interactions may still occur. Displacement from refuge, increased foraging activity, and altered energy allocation are likely to have consequences for survival, fecundity, and thus, fitness. Despite this, co-existence between *G. breviceps* and *N. burrowsius* may be promoted in situations where *G. breviceps* are limited by factors, such as lack of suitable cobble spawning substrate, and environmental stress. Moreover, high habitat heterogeneity and low amounts of cobble substratum may reduce direct interactions with *G. breviceps* through habitat segregation. In total, the series of investigations detailed in this chapter furthers the understanding of competitive interactions between *G. breviceps* and *N. burrowsius*, and indicates factors promoting co-existence.

SECTION 4

Reproduction and recruitment

At top, experimental tanks during an investigation into mudfish reproduction and recruitment, in the presence and absence of macrophytes. At bottom, a backwater area at Te Roto Repo o Tawera where numerous mudfish fry were observed.

Chapter 9.

The influence of environmental conditions on spawning timing and fecundity trade-offs of *Neochanna burrowsius*.

INTRODUCTION

Typically, spawning in freshwater fish is controlled by endogenous circum-annual rhythms, however, exogenous cues can also provide the 'final' trigger (Bye 1984). In some Northern Hemisphere temperate freshwater fish, spawning can occur at precise times of the year, with the commencement of spawning often varying by only one week each year (Bye 1984). This likely reflects the relative predictability of seasonal change experienced in many Northern Hemisphere temperate regions, a characteristic which contrasts with the unpredictable New Zealand situation (Winterbourn et al. 1981). In New Zealand, climatic phenomena, such as the El Niño Southern Oscillation (ENSO), result in unpredictable seasonality (Winterbourn et al. 1981; Townsend 1989). Consequently, commencement of spawning in Galaxiidae, a widespread Southern Hemisphere family of fish, is highly variable (Allibone & Townsend 1997b; Allibone & Caskey 2000). The reproductive biology of many New Zealand freshwater fish species is poorly understood, especially regarding the cues that initiate spawning (Allibone & Caskey 2000). Some studies have found few correlations between environmental factors and the commencement of spawning, other than temperature (e.g. Benzie 1961; Cadwallader 1976; Bonnett 1992; Moore et al. 1999). However, in several well studied diadromous species, discrete environmental cues are important. These include the association of spawning with lunar/tidal cues in inanga (*Galaxias maculatus*; McDowall 1988), and the incidence of spawning during spates in koaro (*G. brevipinnis*; Allibone & Caskey 2000), banded kokopu (*G. fasciatus*; Mitchell & Penlington 1982), and shortjaw kokopu (*G. postvectis*; Charteris et al. 2003).

The timing of reproduction is especially important in species that spawn in spring, yet occupy habitats prone to drying up in summer, such as Canterbury mudfish (*Neochanna burrowsius*; Eldon 1979a), the focus of this study. While early spawning is inhibited by low temperature,

spawning too late may expose *N. burrowsius* eggs and/or fry to conditions of low dissolved oxygen and desiccation (Eldon 1979a). Various spawning dates have been documented for *N. burrowsius* (Cadwallader 1975a; Eldon 1979c). Generally, wild populations commence spawning in late winter with activity peaking in early spring and continuing until late spring (McDowall 1990). However, captive fish and translocated populations have been found to delay spawning, and in some cases have completely failed to spawn (Cadwallader 1973; Eldon 1993). Thus, sensitivity of *N. burrowsius* to suitable spawning conditions may limit populations despite the considerable hardiness of adults (Eldon 1993).

Various environmental cues may influence successful spawning in *N. burrowsius*. These include changes in water temperature, increasing photoperiod, spates, dissolved oxygen levels, and the availability of spawning substrata (Eldon 1979c, 1993). Increases in water temperature and photoperiod trigger spawning in most temperate fish and are likely to influence *N. burrowsius*. Further, Cadwallader (1973) proposed that rainfall or an increase in water level was a necessary stimulus to initiate spawning in *N. burrowsius*. He observed that *N. burrowsius* held in outdoor aquaria spawned after a period of heavy rain, whereas fish held concomitantly indoors did not, despite indoor fish being maintained at higher temperatures. Spates allow spawning in some species of *Galaxias* to occur on temporally inundated habitat, increasing subsequent protection of eggs from aquatic predators (Allibone & Caskey 2000). Such a cue is also potentially important in *Neochanna*, as brown mudfish (*N. apoda*) deposits eggs terrestrially, immediately after the onset of rains following seasonal habitat drying (Eldon 1971, 1978). Although, terrestrial spawning in *N. burrowsius* was initially suggested (Cadwallader 1975a), further investigations into egg survival indicated that terrestrial placement was likely to be unintentional (Eldon 1979a). However, the occurrence of eggs above the water surface is likely if spawning occurs at high water levels, which subsequently subside.

Dissolved oxygen concentration is likely to be important in determining favourable spawning conditions. *N. burrowsius* is often found in habitats that experience hypoxia (Eldon 1979a), and the intensity and extent of episodes of oxygen stress may determine recruitment success. Adult *N. burrowsius* have behavioural and physiological adaptations enabling tolerance to hypoxia (Meredith 1981, 1985), but these features may not be fully developed in early life stages. Eldon (1979c) suggested that low dissolved oxygen affects embryo development and egg mortality rates in *N. burrowsius*. It has been widely demonstrated that low dissolved oxygen concentrations

adversely affect early development in freshwater fish species in a variety of ways, including the induction of deformities, inhibition of growth, premature hatching, and mortality (Siefert et al. 1973; Guma'a 1978; Cloud et al. 1988; Latham & Just 1989; Wiegand et al. 1989; Bonner et al. 1998; Viljanen & Koho 1991; Schiemer et al. 2003). Adverse environmental conditions can also influence fecundity and the trade-off between egg number and size, which may further affect recruitment and survival of early life stages (Bagenal 1969a). Thus, it is likely that fish will avoid spawning during such conditions.

In this study I investigated the proximate environmental cues controlling spawning in *N. burrowsius* and their influence on egg number and size. The response of gravid *N. burrowsius* to manipulations of water level fluctuations and dissolved oxygen concentrations were tested in experimental tanks. I hypothesised that these manipulations represented potential cues responsible for initiating and suppressing spawning, respectively. Post spawning egg number and size were compared between dissolved oxygen and water level treatments, to assess reproductive plasticity in response to manipulated environmental conditions. Finally, patterns in egg size and development were compared between wild populations experiencing different environmental conditions to assess the general applicability of the experimental findings.

METHODS

Tank experiments

To investigate the influence of dissolved oxygen and water level fluctuations on the spawning of *N. burrowsius*, an experiment was conducted during 2000; using twelve 750 litre cattle troughs located outdoors (see Chapter 8 for further details). Tanks contained a diverse invertebrate prey community, and were supplemented daily with approximately 1 g per tank of commercially available frozen Chironomidae. Tanks were stocked at a density of six *N. burrowsius* per tank (3 male, 3 female) as previous experiments (Chapter 8) indicated that this density allowed reasonable fish growth. Mean male *N. burrowsius* total length was 87 ± 1.7 mm TL (mean ± 1 SE), and females were 95 ± 2.2 mm TL (mean ± 1 SE). Fish were anaesthetised with 2-phenoxyethanol before being sexed externally (Figure 9.1), by examination under x16 magnification, following the guidelines of Cadwallader (1973) for sexing Canterbury galaxias (*Galaxias vulgaris*).

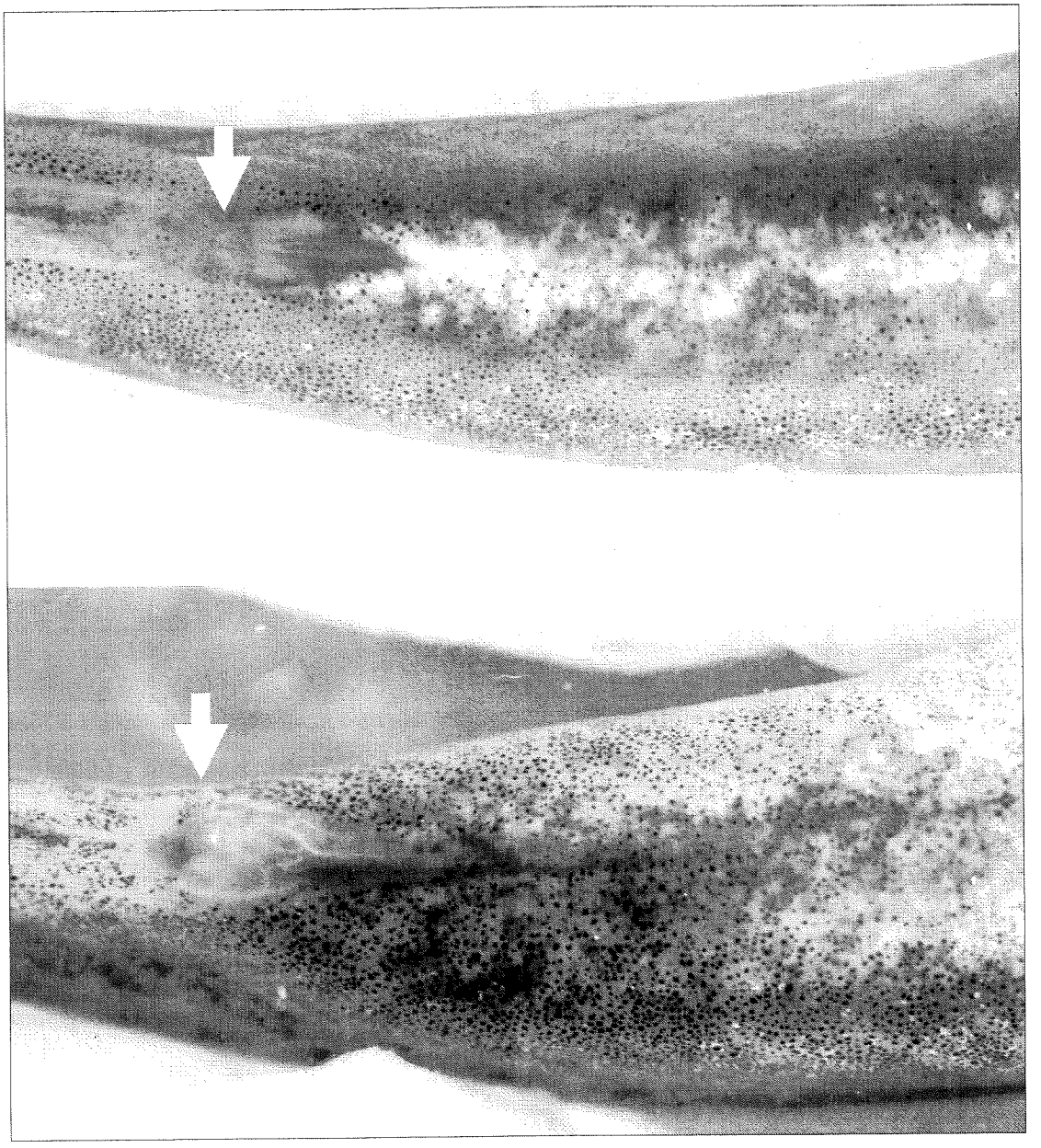

Figure 9.1. Male *N. burrowsius* (top) possess a papilla (arrowed), at the tip of which is the genital opening. Females (bottom) lack a papilla and the genital region is much more bulbous (arrowed) than in males. Note also the whitened abdomen of the ripe male due to milt.

Sexes were kept separate until fish showed obvious signs of spawning readiness, such as flowing milt in males and developed eggs being visible through the abdominal skin in females. Mature fish of both sexes were randomly allocated to each tank, with it being anticipated that fish would spawn shortly thereafter.

N. burrowsius were added to experimental tanks which were randomly allocated a combination of the two treatments in a factorial design. To simulate a water level rise caused by rainfall, six of the tanks were maintained at half their normal water volume for a period of one week subsequent to fish addition; then during a rain event, these tanks were refilled to a level equivalent to the six control tanks, which experienced no water level fluctuation. Conducting this treatment during a rainstorm was intended to imitate the barometric and other associated environmental cues associated with a small spate. This manipulation was applied on 18 and 26 August 2000. Tanks took approximately one and a half hours to fill and were slowly drained to half-full over several days, between applications. Mean water level fluctuation in manipulated tanks was 14 ± 0.5 cm (mean ± 1 SE); with the maximum depth of tanks being 40 ± 1.0 cm (mean ± 1 SE).

In six of the tanks (three per water level treatment), dissolved oxygen concentrations were manipulated by bubbling nitrogen gas through the water to reduce the partial pressure of oxygen, whereas compressed air was bubbled through the six control tanks (Figure 9.2). The rate of gaseous flow to each tank was controlled using adjustable hose clips and maintained at a consistently low level to minimise turbulence. Dissolved oxygen concentrations were measured daily with an YSI Model 95 probe. Significantly different dissolved oxygen concentrations were maintained between treatments throughout the experiment (One-way ANOVA: $F_{(1, 10)} = 68$, $p < 0.001$), although daily levels fluctuated with environmental conditions; being on average 12.8 ± 0.3 mg/l (mean ± 1 SE), and 9.2 ± 0.7 mg/l (mean ± 1 SE) in air bubbled tanks (high oxygen level) and nitrogen bubbled (low oxygen level) tanks, respectively. This treatment was applied continuously for 23 days. Following cessation of the manipulation, dissolved oxygen concentrations were identical in all tanks within 24 hours.

Water temperature was monitored with a data logger placed in one tank and minimum/maximum thermometers placed in all tanks. Information on lunar phase was obtained using LunarPhase Version 2.50 software (Nugent 1999).

Figure 9.2. Experimental setup used to investigate the influence of environmental conditions on spawning of *N. burrowsius*. Pressurised gas cylinders (left) were used to bubble gas through water to manipulate dissolved oxygen levels in outdoor tanks (right).

N. burrowsius typically spawn on macrophytes, thus two bunches each of water milfoil (*Myriophyllum* sp.), and watercress (*Rorippa* sp.), were placed in each tank. To assess if spawning had occurred, one bunch of each plant species was examined daily for the presence of eggs, in a shallow white tray. Additional five minute egg searches of the whole tank were conducted before concluding that spawning had not occurred. If spawning had occurred, one bunch of each macrophyte species was placed in a water filled container and further examined in the laboratory. Numbers of eggs on macrophyte bunches were recorded and the diameter of eggs measured under x35 magnification with a calibrated, graduated eye piece.

Statistical analysis

Factorial ANOVA was used to test the influence of the two experimental manipulations, oxygen concentration (high and low), and water level (stable and fluctuating) on the timing of *N. burrowsius* spawning. Differences in the timing of spawning were tested by ranking spawning

dates across all treatments, generating a normally distributed dependent variable (Shapiro Wilks $W = 0.9$, $p = 0.6$). Regression analysis was used to test the relationship between the number of eggs collected and egg diameter. All analyses were conducted in Statistica 6.0 (StatSoft Inc. 2001).

Field survey

To investigate the timing of spawning in wild *N. burrowsius* populations, eggs were collected from four sites on the Canterbury Plains, being Te Roto Repo o Tawera, Hororata Spring, St Andrews Drain and Dog Kennel Stream, during the period 27 – 31 August 1999 (See Chapter 1 for further description of study sites). It was hypothesised that if spawning was highly synchronised within sites, eggs in the field would be of similar developmental state. Furthermore, if spawning was influenced by temperature, *N. burrowsius* in Mid Canterbury sites would be expected to spawn before those in South Canterbury, and be indicated by further developed eggs. As *N. burrowsius* typically spawns on macrophytes (Eldon 1979c), macrophyte samples were randomly gathered from sites, transported with water in plastic bags, and examined while plant material was fresh. Eggs were washed from plant material over a 0.5 mm sieve. They were then preserved and dehydrated using Carnoy's fixative (75 % absolute ethanol, 25 % glacial acetic acid); before being cleared with, and stored in, methyl salicylate. Differences in the weights of eggs between sites were investigated by weighing preserved eggs. The chorion of each egg was removed under a microscope to eliminate any accumulated debris so that a more accurate embryo weight could be obtained. Eggs were then separated into three developmental stages (see Appendix 1), dried at 40 °C for 24 hours and stored in a desiccator. A Mettler Toledo microbalance was used to weigh batches of embryos of the same developmental stage to the nearest 0.001 mg. One-way ANOVA was used to test differences in egg dry weight between sites, with natural log transformed data.

RESULTS

Tank experiments

Spawning behaviour was observed during the experiment, which on one typical occasion occurred at 17:30 New Zealand Standard Time (NZST) when the water temperature was 11 °C. Usually, four of the six fish present were involved, suggesting that the group comprised one female being pursed by all three males present in the tank. Initially, the behaviour was conspicuous, with fish swimming through macrophyte patches very energetically. They gave the appearance of chasing one another, rubbing and flicking their bodies and tails against each other and the macrophytes. However, group-spawning did not appear to occur and large amounts of milt were not observed, as occurs after *G. maculatus* group-spawning (McDowall 1990). Instead, the group thinned and apparently co-ordinated mating behaviour was observed between the two leading fish. This involved frequently crossing over one another's body mimicking one another's movements (Figure 9.3). Gay (1999) also observed a pair of pre-spawning *N. burrowsius* moving in unison at the water surface.

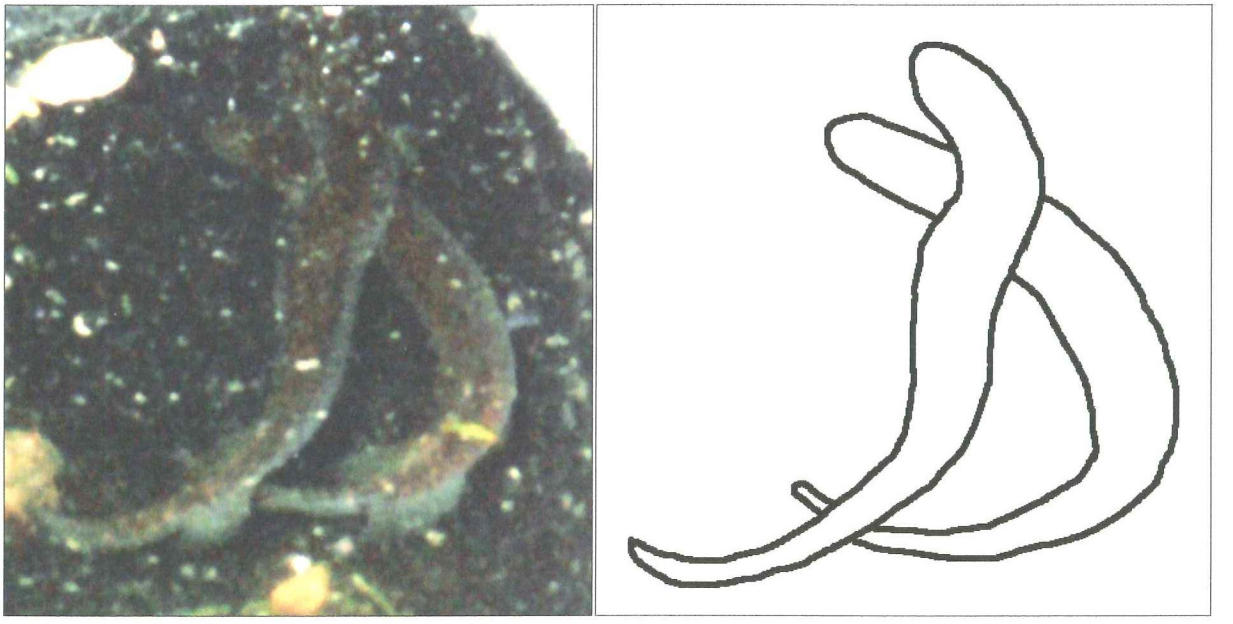

Figure 9.3. Spawning behaviour in *N. burrowsius* involved synchronised movements, such as this pair which repeatedly crossed over each other closely (left = original photograph, and right = a trace of the fish taken from the photograph).

Environmental cues

Unexpectedly – considering the apparently advanced gravid stage of females and presence of readily flowing milt in males – spawning did not occur until 23 days after the sexes were placed together. Moreover, spawning occurred immediately after cessation of the oxygen concentration manipulation, which involved bubbling gases through the tanks, implying that this activity/disturbance had delayed spawning (Figure 9.4).

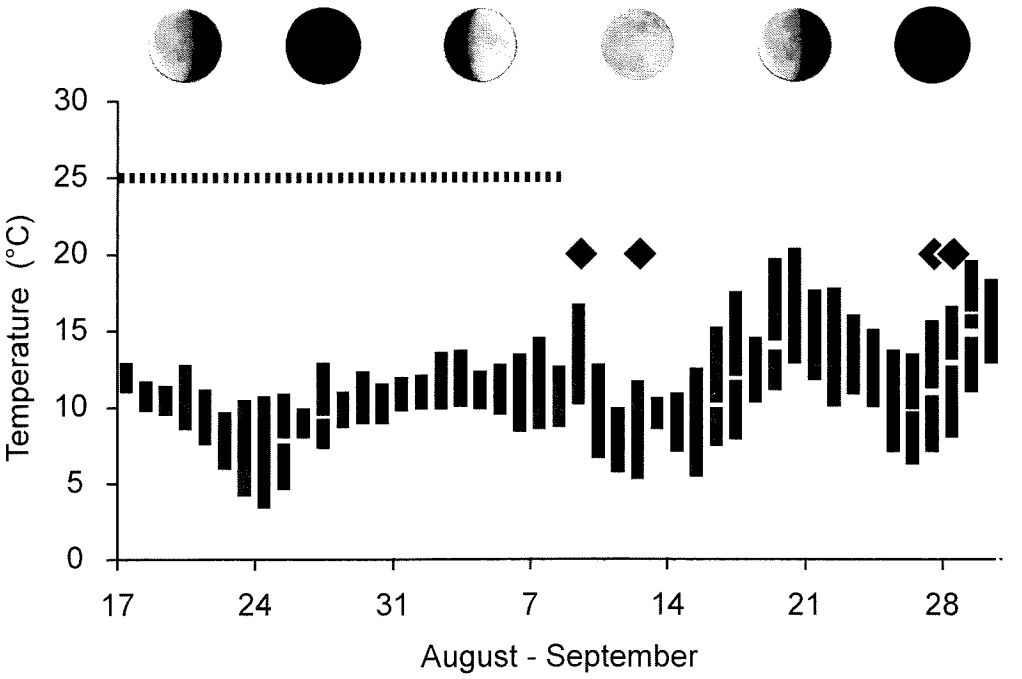

Figure 9.4. Spawning timing, water temperature and lunar phase during investigations of the influence of environmental cues on the spawning of *N. burrowsius*. The dashed line at top left indicates the period of experimental manipulation involving bubbling of gas through the tanks. Vertical blocks indicate water temperature ranges. Images at top indicate waxing and waning of the lunar phase. Diamonds represent initial spawning events.

Initial spawning occurred in two tanks, after which there was a two week period before any further spawning occurred. By 30 September 2000, 44 days after the experiment was initiated, evidence of *N. burrowsius* spawning had been found in only half of the tanks (Figure 9.4). Spawning was inhibited most in tanks that had low dissolved oxygen and water level fluctuation

manipulations as spawning did not occur until 10 October 2000 (Figure 9.5, Table 9.1). The treatment that attempted to simulate a 'spate' by raising water levels did not trigger spawning as hypothesised. However, it is likely that any response to this cue was suppressed by the turbulence and noise caused by bubbling gas through the tanks. Nonetheless, both experimental manipulations influenced the timing of subsequent *N. burrowsius* spawning (Table 9.1). Females that had experienced low dissolved oxygen and stable water levels tended to spawn slightly before others. Whereas overall, females that had experienced fluctuations in water level delayed their spawning to a much greater degree (Figure 9.5).

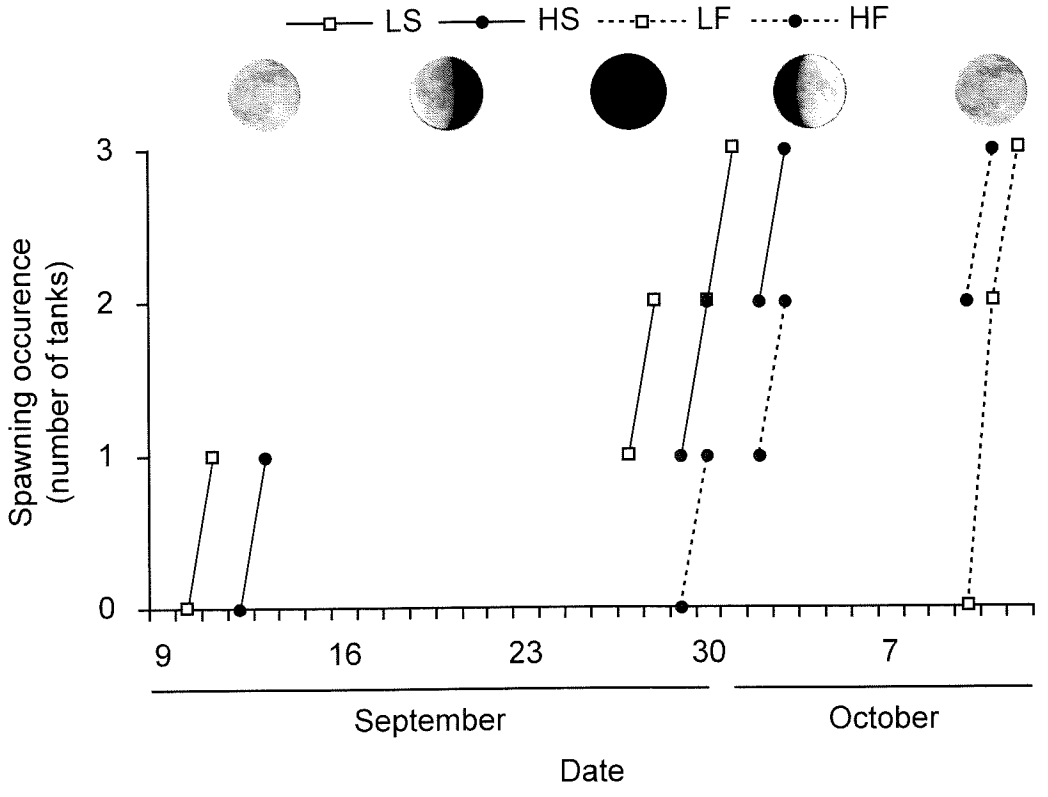

Figure 9.5. Cumulative number of *N. burrowsius* spawning events in each tank in relation to the manipulations of dissolved oxygen and water levels. Treatment codes: L, tanks with low dissolved oxygen due to bubbled nitrogen; H, tanks with high dissolved oxygen due to bubbled air; S, stable tanks with no water level manipulation; F, tanks which experienced water level fluctuations. Images at top indicate waxing and waning of the lunar phase.

Table 9.1. Results of factorial ANOVA testing the influence of environmental manipulations on the time of spawning (ranked date) in *N. burrowsius*. Dissolved oxygen concentration and water level in tanks were manipulated.

Source	df	MS	F-ratio	*p*-value
Oxygen level	1	225	42.8	< 0.001
Water level	1	75	13.1	0.007
Oxygen x Water	1	16	2.9	0.13
Error	9	6		

An important finding was the occurrence of peaks in spawning activity, approximately two weeks apart. These peaks in spawning could be loosely associated with an increase in water temperature above approximately 15 °C (Figure 9.4). However, temperature cannot be the sole factor that determines spawning, as it did not occur when the temperature rose around 20 September (Figure 9.4). Lunar cycles involve fortnightly patterns in luminosity and gravitational influence and comparison of the occurrence of full and new moons and *N. burrowsius* spawning suggest an association (Figure 9.5).

Eggs

A negative relationship between the number of eggs collected in tanks and their size was found across experimental treatments, (R^2 = 0.64, $F_{(1, 6)}$ = 10.9, p = 0.016; Figure 9.6), indicating a trade-off between reproductive allocation to egg size versus number.

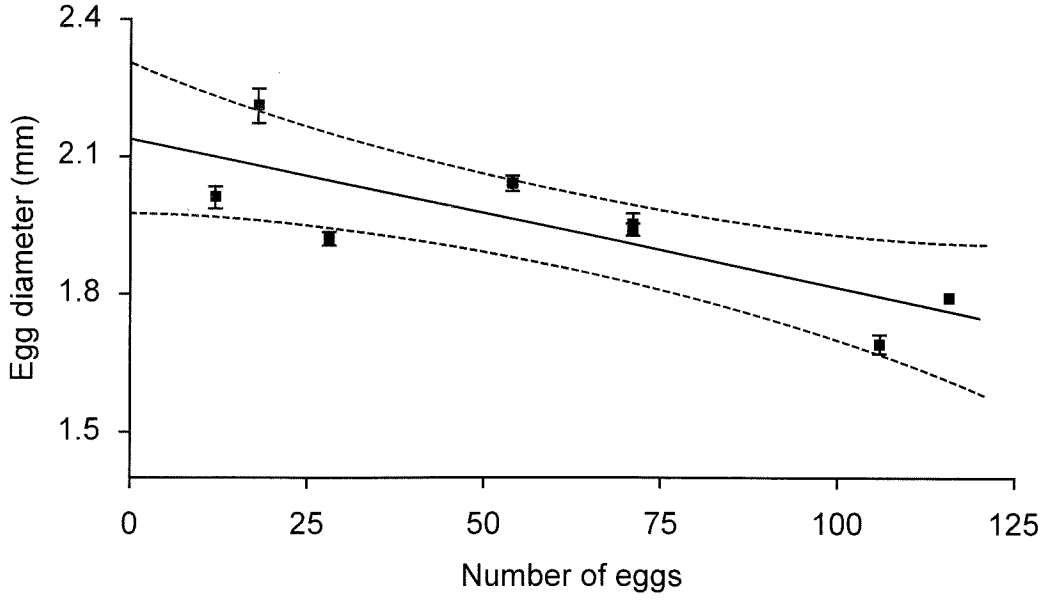

Figure 9.6. Number and diameter (mean ± 1 SE.) of newly spawned eggs found on half of the available spawning substratum within eight tanks (two from each treatment). 95 % confidence levels for the slope of the regression line are indicated by dotted lines.

This trade-off was also influenced by the environmental conditions experienced by *N. burrowsius* prior to spawning because both water level fluctuations ($F_{(1, 307)} = 215$, $p < 0.001$) and dissolved oxygen concentrations ($F_{(1, 307)} = 9$, $p < 0.01$) influenced egg diameter, however there was no significant interaction between treatments. Larger eggs were found in stable water level conditions with low dissolved oxygen, whereas smaller eggs were found in those tanks that experienced fluctuating water levels (Figure 9.7). Resources and conditions experienced by all females, before the spawning experiment when gonads were maturing, were similar and not related to experimental treatments. Consequently, any subsequent reproductive responses and trade-offs associated with the environmental treatments occurred within the same energetic constraints. Moreover, differences must have arisen from exposure to the experimental treatments during the relatively late stages of gonad development. There was no significant difference in the maximum temperature of tanks between treatments during the experiment. Neither were there significant differences in number of eggs occurring on the two different macrophyte species.

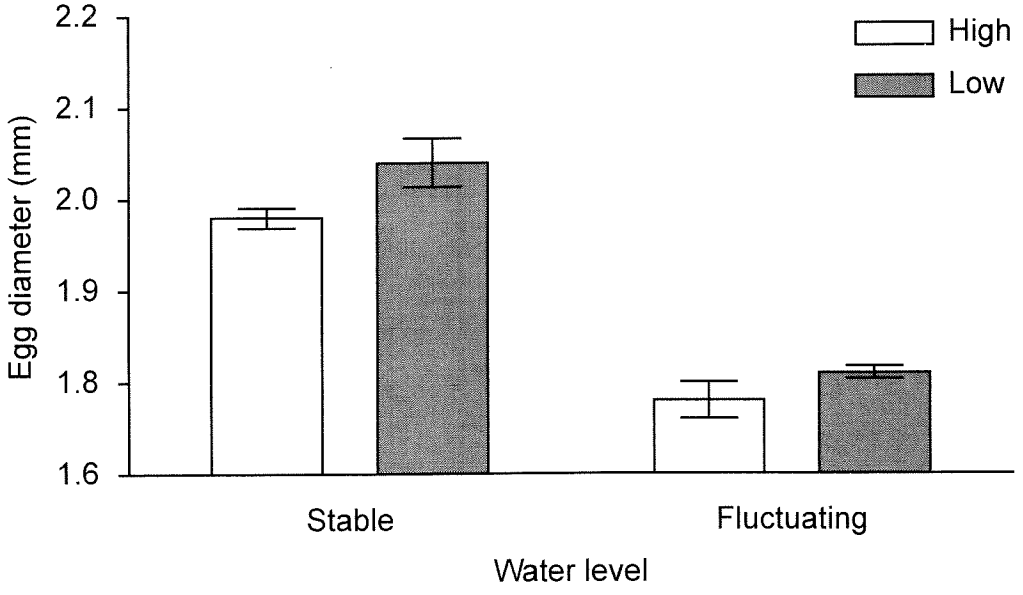

Figure 9.7. Diameter of eggs (mean ± 1 SE) collected from eight tanks (two from each treatment). Treatments were applied in a factorial design, being either stable or fluctuating water level, and high or low dissolved oxygen concentration.

Field survey

Egg weight

N. burrowsius eggs collected in the field were weighed to investigate whether differences in female investment to embryos could be related to environmental conditions prevalent at different sites. Larger field collected eggs appeared to weigh more, however, preservation resulted in egg shape distortion, and thus direct comparisons with the experimental results could not be made. Nevertheless, egg weight differed significantly between sites (One-way ANOVA: $F_{(3, 40)} = 4.4$, $p = 0.009$; Figure 9.8), especially between those in Mid (Te Roto Repo o Tawera and Hororata Spring), and South Canterbury (St Andrews Drain and Dog Kennel Stream). Egg weight did not differ between the three stages of egg development identified (One-way ANOVA: $F_{(2, 40)} = 1.8$, $p = 0.18$), similar to the findings of Davenport & Lönning (1980), for Atlantic cod (*Gadus morhua*).

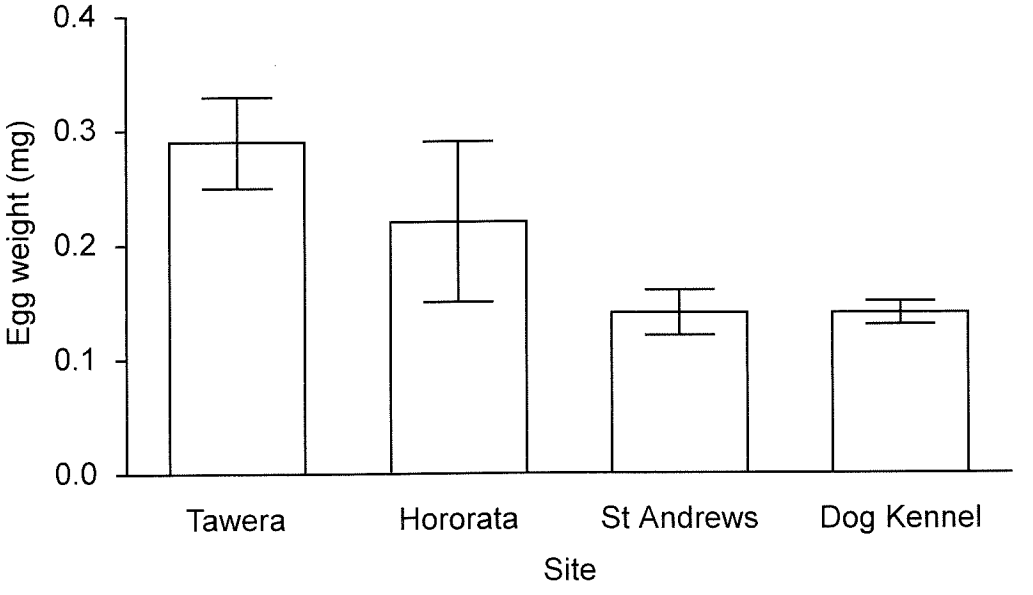

Figure 9.8. Embryo weight for eggs with chorion removed (mean ± 1 SE), collected during egg surveys at Te Roto Repo o Tawera, Hororata Spring, St Andrews Drain and Dog Kennel Stream during the last week of August 1999.

Egg stage

The developmental stage of eggs provides information on general spawning times and an indication of recent water temperatures. Eggs collected from Te Roto Repo o Tawera had developed further than eggs from all other sites (Table 9.2). Furthermore, fry were present at this site during sampling, despite sampling there occurring two days before other sites. Overall, however, there was little evidence that spawning had occurred earlier at northerly than southerly sites. Instead, the pattern between sites implied that fish at perennially flowing sites (Te Roto Repo o Tawera and St Andrews Drain) spawned before fish at sites that had experienced drying during the previous summer and autumn (Hororata Spring and Dog Kennel Stream). Interestingly, at Te Roto Repo o Tawera, where most eggs were found, nearly twice as many eggs were at developmental stages I and III, than at stage II, with it taking approximately two weeks for eggs to develop from stage I to stage III at 15 °C (Appendix 1). Samples were collected at Te Roto Repo o Tawera on 27 August 1999, one day after the occurrence of a full moon. This could indicate that the spawning peaks observed during tank experiments may also occur to some extent in the wild, further supporting the association of *N. burrowsius* spawning with a semi-lunar cycle.

Table 9.2. Number of locations where macrophyte samples containing eggs were found at Te Rcto Repo o Tawera, Hororata Spring, St Andrews Drain, and Dog Kennel Stream in the period 27 – 31 August 1999. Percentage of total samples containing eggs, total number of eggs found, and where possible their developmental stage are given. See Appendix 1 for description of egg stages I – III.

	No. locations	% samples	Total eggs	Stage I	Stage II	Stage III
Tawera	14	37	167	76	29	51
Hororata	2	14	15	11	0	0
St Andrews	3	15	42	29	5	6
Dog Kennel	3	13	34	17	13	0

DISCUSSION

Spawning delay

The results of this study demonstrate that *N. burrowsius* readily delays the commencement of spawning if conditions are unsuitable. Eldon (1979c) found that spawning in wild populations started in July, whereas in the field I found fry and eggs at various developmental stages in August. Furthermore, the delay in spawning until September and October shown in tank experiments demonstrates an ability to postpone spawning, despite apparent spawning readiness. Long delays between ovulation and oviposition are common in species with elaborate reproductive behaviours, such as nest building and mate selection (Lam et al. 1978). However, *N. burrowsius* do not form egg nests (Eldon 1979c). Further, Cadwallader (1975a) suggested that *N. burrowsius* had less precise fertilisation processes than other species of *Neochanna* due to their greater fecundity. Thus, the observed delay in spawning was likely due to disturbances applied during experimental manipulations, mainly in the form of turbulence from gas bubbles. An ability to delay spawning is likely to have arisen because of the unpredictably disturbed and frequently inhospitable habitats of *N. burrowsius* (Eldon 1979a). Spawning delay has also been noted in *N. diversus*, which also occurs in hydrologically disturbed habitats (Perrie 2004).

Retention of ovulated eggs in the ovarian cavity for considerable periods has been related to the longevity of the post-ovulatory corpus luteum (Lam et al. 1978). Although, little information is available on the reproductive physiology of *Neochanna* species, Davidson (1949) found that *N. apoda* differed from *G. maculatus* and *Salmo* spp. by having developed a closed ovisac, unlike the latter genus whose ova fall into the cavity of the abdomen before excretion. Such physiological modifications could conceivably improve egg retention and viability by providing a means of retaining eggs and maintaining them in suitable conditions, e.g. with hormones.

Although the ability to wait until conditions are suitable could improve survival chances of eggs and fry, long delays in spawning could also be detrimental. In a study investigating the effects of delayed spawning in Pacific herring (*Clupea harengus pallasi*), long delays induced by captivity, of 2 – 3 months beyond normal spawning dates resulted in progressive loss of egg and larval viability (Hay 1986). Spawning postponement may also affect adults. For example, in cases of delayed spawning in threespine stickleback (*Gasterosteus aculeatus*) eggs became 'overripe' and females no longer responded to male courtship (Lam et al. 1978). Further, Perrie (2004) observed a female black mudfish (*Neochanna diversus*) that was heavily gravid and had difficulty swimming and maintaining equilibrium. Long delays may also lead to adult mortality as indicated by the work of Duffy (1996) who found dead, ripe *G. brevipinnis* in Apias Creek, Ruahine Range, and implied that inhibition of spawning due to the absence of a spate cue may have been a contributing factor. The gonads of these fish, in particular the females occupied almost the entire body cavity. Deaths attributed to a failure to spawn have also been observed in captive *N. diversus* and Northland mudfish (*Neochanna heleios*; Perrie 2004). Thus, consequences such as pressure on other organs and constraints on stomach size that impose energetic restrictions may occur if spawning delay is prolonged. Avoidance of these consequences through atresia and resorption of eggs may be possible and has been suggested in roundhead (*G. anomalus*), and flathead galaxias (*G. depressiceps*), which failed to spawn in laboratory trials (Allibone 1997). However, atresia is most commonly related to nutritional deprivation prior to ovulation (Rideout et al. 2000).

In seasonally hypoxic or desiccating habitats, early spawning would be advantageous in allowing greater fry development before the onset of summer water level decline and possible hypoxia. However, experimental and field data do not support the hypothesis that *N. burrowsius* living in intermittent habitats prone to hypoxia spawn earlier to improve recruitment success. The

occurrence of low dissolved oxygen and habitat desiccation during summer is likely to impose stress and energetic demands on adults (Chapter 7). Further, the occurrence of emersion may delay reproductive readiness by diverting energy to maintaining homeostasis, and somatic demands, rather than gonadal development. Thus, the behavioural plasticity in spawning demonstrated in experiments, although potentially advantageous, may not over-ride constraints imposed by resource limitations in the wild. Such limitations have been implicated in the inhibition and total spawning failure of other fish species, for example redbelly tilapia (*Tilapia zillii*), in which overcrowding in captivity inhibited spawning by suppressing sex steroids (Coward et al. 1998). Similarly, food limitation was implicated in the spawning failure of *G. morhua* from Newfoundland in 1999 (Rideout et al. 2000).

Spawning cues

Increasing water temperature frequently influences the decision of fish to spawn as it is a readily-detected indicator of the advancing season and certain thresholds have been shown to trigger spawning in some species (Bye 1984; Koya & Kamiya 2000). In *N. burrowsius*, short term increases in temperature did not necessarily result in spawning. Unexpectedly, lunar phase appeared most closely associated with the periodic spawning events observed in *N. burrowsius*. Lunar synchronicity is rare in freshwater species but not without precedent, and has been recorded in lotic and lentic species such as Cichlidae and *Tilapia* spp. (Schwanck 1987; Nakai et al. 1990). Combined observations of spawning dates for *N. burrowsius* from tank experiments in 2000 (this chapter) and 2001 (Chapter 10), as well as dates recorded by Cadwallader (1973), and Eldon (unpublished data) indicate spawning tended to occur just after a new moon and just before a full moon (Figure 9.9). Thus, many more incidences of spawning have been recorded during a waxing, than a waning moon.

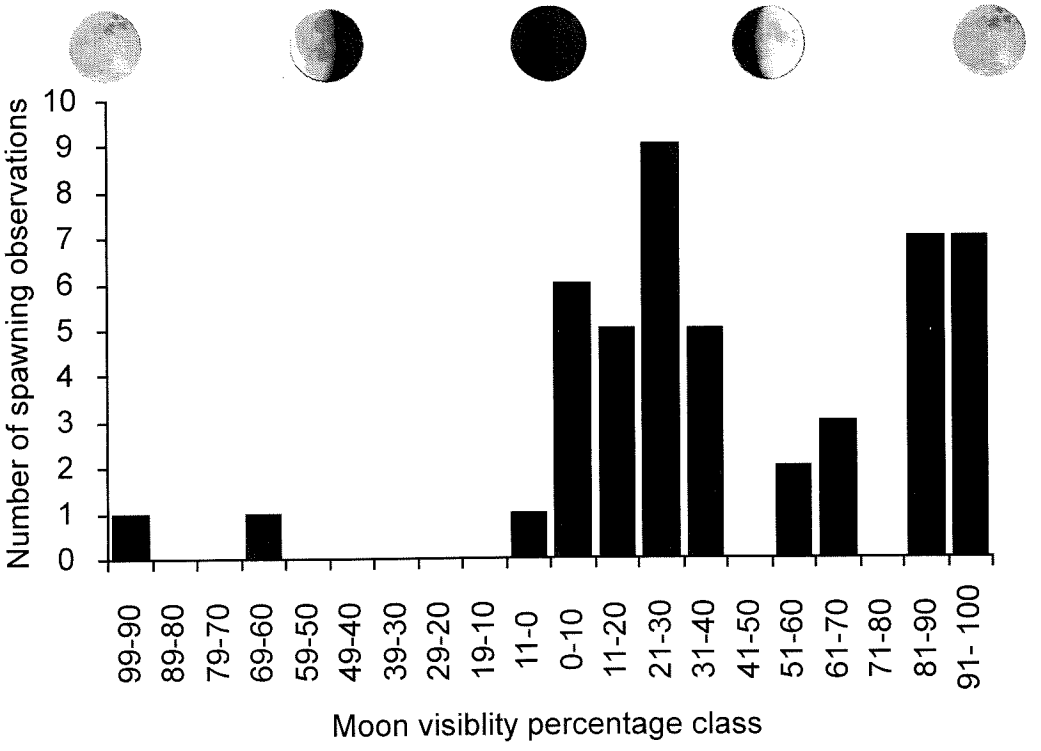

Figure 9.9. Percentage of moon visible during accurately recorded spawning incidences. Information on the frequency of spawning in relation to the waxing and waning of the moon was compiled from Cadwallader (1975a), Eldon (unpublished data) and the present study (this chapter and Chapter 10).

Spawning activities were also concentrated during lunar waxing in eight species of Cichlidae in Lake Tanganyika (Nakai et al. 1990), and in spotted tilapia (*Tilapia mariae*) in the Ethiop River, Nigeria (Schwanck 1987). Two evolutionary mechanisms have been suggested to explain the link between lunar cycles and reproductive behaviour in these freshwater fish. Lunar cycles may be used as a cue for synchronising reproductive readiness, because synchronized spawning may help maintain a wide genetic mix in the population, by ensuring high participation (Bye 1984). As the genetic diversity of *N. burrowsius* is extremely low (Davey et al. 2003), processes increasing genetic mixing would be highly advantageous. Furthermore, modelling studies suggest that cyclic reproduction cues would have adaptive significance in situations of low population size, reducing the likelihood of extinction (Omori 1995).

An alternative hypothesis is that the apparent synchronicity relates to moonlight levels rather than direct gravitational cues. New and full moons are approximately gravitationally equivalent but

have different luminosities. Many of the freshwater species for which lunar cycles are associated with reproduction are diurnal, and show some level of parental care. Within the waxing period they tend to spawn just before the full moon when high levels of moonlight allow enhanced guarding efficiency against nocturnal predators (Schwanck 1987; Nakai et al. 1990). The situation is different in *N. burrowsius*, which was more likely to spawn directly after the new moon. *N. burrowsius* is largely nocturnal and its activity is not limited by low light intensity. However, it has been shown that nocturnal species frequently reduce their activity during periods of bright moonlight (Lima & Dill 1990). Thus, the high incidence of spawning during the waxing of the moon implies that *N. burrowsius* may prefer dark nights on which to spawn. Spawning is a potentially conspicuous activity, so such timing would help fish avoid visual predators. The activities of several other nocturnal New Zealand freshwater fish species are also related to phases of the moon, and imply a response to moonlight. *Anguilla* species, for example, are more likely to commence downstream spawning migration, and the return of their glass eels occurs, during the last quarter of the moon, rather than during a full moon (McDowall 1990; Jellyman & Lambert 2004). However, such avoidance of moonlight does not totally explain the spawning patterns observed in *N. burrowsius*, as spawning also occurred just prior to the full moon.

Strong synchronisation with lunar cues has mostly been described in marine and migratory species and is related to tidal patterns (McDowall 1969, 1988; Taylor 1984). However, it is possible that the synchronisation of spawning with lunar cycles in *N. burrowsius* is a behavioural remnant indicative of an ancestral origin involving a marine migratory phase. Indeed, the Australian congener, Tasmanian mudfish (*N. cleaveri*), still retains a diadromous life cycle (Andrews 1991). McDowall (1997a) considered this behaviour was a primitive feature reflecting an ancient Salmoniform relationship. Another New Zealand example of lunar synchronicity is found in the diadromous *G. maculatus*, which spawns during spring tides when tidal height allows access to terrestrial spawning habitat (Burnet 1965; McDowall 1968, 1969, 1988). Although this behaviour seems to be a relatively straight forward response to tidal height, rather than the proximate lunar phase, adults occupy freshwater habitats beyond the tidal influence. Despite this, their downstream spawning migration is also thought to be influenced by lunar cycles (Burnet 1965). Being of the same family, the ability of *Galaxias* and *Neochanna* to perceive gravitational or otherwise correlated cues may have a common physiological basis, although its mechanism remains a mystery (McDowall 1969). Irrespective of the suggested

relationship with lunar phase, the occurrence of the distinctive peaks or 'waves' of spawning activity in *N. burrowsius* was unexpected. Nevertheless, this pattern has been noted in other fish species, such as *C. harengus pallasii* (Hay 1986).

Extended spawning

Once spawning commences in *N. burrowsius* it occurs over an extended period, usually three months (Eldon 1979c). This is in contrast to *Galaxias* species, such as *G. maculatus* (Benzie 1968), and non-migratory galaxiids in the Taieri River system (Allibone & Townsend 1997b), which appear to spawn over a period of approximately one month. However, the extended spawning of *N. burrowsius* is similar to that of *N. apoda*, which breeds in most months of the year in habitats with perennial water (Eldon 1978). In the present experiment, despite the lengthy initial delay, spawning of *N. burrowsius* continued for nearly two months. Furthermore, the wide range of developmental stages in eggs collected from the field, as well as the presence of fry during egg collection periods supports the occurrence of an extended spawning period.

An extended spawning period may be a 'bet-hedging' strategy to maximise the chances that at least some off-spring will survive in a fluctuating and unpredictable environment (Medeiros & Maltchik 2000; Spranza & Stanley 2000). Environmental constraints often place strong selection pressures on reproductive styles, as in the central stoneroller (*Campostoma anomalum*) in which divergent reproductive styles were observed at sites with differing hydrological regimes. Specifically, prolonged spawning was found in fluctuating habitats, despite sites being within 1 km of each other (Spranza & Stanley 2000). In New Zealand the success of the non-migratory upland bully (*Gobiomorphus breviceps*) in streams that frequently dry up has been attributed to a prolonged spawning season (McDowall & Eldon 1997). However, these examples of extended spawning relate to batch spawning species, where prolonged, repeated spawning increases the chance of an individual to produce progeny. In contrast, *Neochanna* species spawn only once per year (Eldon 1979c). However, extended or variable spawning in response to the environment should promote the persistence of populations in the highly fluctuating habitats often occupied by *N. burrowsius*.

Fecundity and egg size

In experimental manipulations of tank conditions, a negative relationship was found between the number and size of eggs produced by *N. burrowsius*. There is a strong tendency for such trade-offs in fish, with both inter- and intra-specific patterns observed due to constraints on energy and body volume available to the gonads (Blaxter 1969). Both maternal and environmental factors have been recognised as determining these trade-offs. For example, egg size was attributed to the growth history of female brown trout (*Salmo trutta*), with faster growing fish producing many smaller eggs (Bagenal 1969a; Lobón-Cerviá et al. 1997). A similar pattern was observed in white-spotted charr (*Salvelinus leucomaenis*), with females that grew fast in their first year producing smaller eggs than those that grew fast in their second year (Morita et al. 1999). However, commonly observed correlations with maternal characteristics, including female size (Lobón-Cerviá et al. 1997; Johnston & Leggett 2002), are unlikely to be directly relevant to *N. burrowsius* as the pre-experimental conditions for growth of fish in my trials were similar and not related to the experimental manipulations to which females were randomly assigned.

Life-history theory suggests that environmental selection will have a strong influence on egg size, and that this in turn will determine fecundity (Johnston & Leggett 2002). Furthermore, theory predicts that large egg size should be selected for under conditions of poor initial habitat quality for fry (Johnston & Leggett 2002). In contrast, very high rates of offspring mortality should favour individuals with high fecundity, thus necessitating small egg size. Thus, the trade-off between fecundity and egg size can be related to the opposing constraints of likely food availability and mortality rate of fry. In the case of Galaxiidae, the larvae of diadromous species suffer high mortality during dispersal to the marine environment, which favours selection for high fecundity (McDowall 1970). Such reasoning can be applied to intra-specific differences as well. For example, Blaxter (1969) found that winter – spring spawning Atlantic herring (*Clupea harengus*) had fewer, larger eggs; an adaptation to food limitation for fry, and low predation rates, whereas, summer – autumn spawners had many more smaller eggs, in response to high mortality, and abundant food resources for fry.

Because yolk and oil volumes are related to egg size, large eggs represent greater maternal investment to individual embryos. Fry hatching from larger eggs are generally longer than those from smaller eggs (Moodie et al. 1989; Viljanen & Koho 1991). Resistance to starvation, and

thus, survival is also typically higher in fry from larger eggs (Bagenal 1969b; Moodie et al. 1989). Hence, high individual investment provides offspring with energy reserves to overcome initially adverse conditions or resource limitations, enabling them to survive longer periods before initial feeding (Viljanen & Koho 1991). Thus, there appears to be little advantage, in terms of fry survival, of producing smaller eggs. However, embryos of Artic charr (*Salvelinus alpinus*) from smaller eggs had faster embryonic development, presumably because attributes related to external feeding developed more quickly to compensate for reduced reliance on yolk (Valdimarsson et al. 2002).

It has been widely assumed that small eggs would be more tolerant of low dissolved oxygen concentrations (Moodie et al. 1989; Johnston & Leggett 2002). This conclusion is based on suppositions of oxygen requirement and surface to volume ratios. However, empirical testing of this assumption has shown that in fact, larger eggs have higher survival rates in situations of reduced dissolved oxygen (Einum et al. 2002). Similarly, exposure of *N. burrowsius* adults to low dissolved oxygen prior to spawning lead to larger eggs being produced. Thus, increased egg size, and thus fry size, is likely to improve chances of fry survival in periodically hypoxic habitat in which food limitation may occur. Furthermore, it has been suggested that producing large eggs with a large volume of perivelline fluid, may be an adaptation whereby egg diameter is increased without increasing specific weight, thus, reducing sinking velocity (Mangor-Jensen et al. 1993). Such a strategy would be advantageous for *N. burrowsius* in hypoxic conditions because the slower an egg sinks the more likely it is to adhere to macrophytes near the water surface, where dissolved oxygen levels are highest. In contrast, *N. burrowsius* produced much smaller eggs in tanks that underwent fluctuations in water level. Thus, the fluctuating water levels in experimental tanks may be 'perceived' as a factor increasing the likelihood of fry mortality, possibly through inadvertent dispersal into unfavourable habitat.

Based on my experimental results it might be expected that large eggs would occur in intermittent sites, which frequently became hypoxic, and small eggs would be found in flowing streams. However, although there were significant differences in egg mass between wild populations, distributional patterns were not straight-forward and appeared to be related more to latitude than hydrological regime. A decrease in egg size with an increase in latitude has been observed widely in fish species and has been attributed to the effect of temperature (Johnston & Leggett 2002). Furthermore, Jonsson & Jonsson (1999) found that high water temperature during

spawning increased the mass of *S. trutta* eggs. They concluded that because of lower efficiencies in the conversion of yolk to body tissue, large eggs would be advantageous at higher water temperatures (Jonsson & Jonsson 1999). Thus, it is likely that factors additional to dissolved oxygen and water fluctuations, such as temperature, are responsible for determining the observed site specific differences in fecundity and egg size observed in *N. burrowsius*.

It is considered that phenotypic plasticity in egg size is an adaptive advantage for organisms breeding in a variable environment (Johnston & Leggett 2002). The results of my experiment suggest that exposure to reduced dissolved oxygen and water volume fluctuation over a two week period was sufficient to elicit a reproductive response in *N. burrowsius*. The mechanism by which females manipulated fecundity and egg size during late development is unknown. However, although the maximum number of eggs is determined early in the ovarian cycle, egg number can be reduced by selectively cutting off blood flow to developing follicles through atretic degeneration (Johnston & Leggett 2002). Although the mechanisms of plasticity in individual egg size may still be uncertain, the occurrence of egg plasticity demonstrates the adaptability, and responsiveness of *N. burrowsius* to its environment. Furthermore, the ability to delay spawning until suitable conditions arise, and the occurrence of an extended spawning period are important characteristics that promote persistence in frequently disturbed habitats.

Chapter 10.

The role of macrophytes in determining recruitment of *Neochanna burrowsius*.

INTRODUCTION

In lentic habitats aquatic macrophytes serve an important role in recruitment, survival, and thus year class strength of phytophillic spawning fish species (Barnett & Schneider 1974; Moxley & Langford 1982; Hoyer & Canefield 1996). Canterbury mudfish (*Neochanna burrowsius*), the focus of this study, is a phytophillic spawner, so macrophytes are a necessary feature of 'optimum' *N. burrowsius* habitat (Eldon 1993). Macrophytes are subject to intensive management in most agricultural waterways (Hudson & Harding 2004). However, these drains and races are often all that remains of *N. burrowsius*' wetland habitat. Whether such management practices limit *N. burrowsius* populations could depend on the extent to which *N. burrowsius* require the presence of abundant aquatic macrophytes as spawning substrate.

N. burrowsius scatters its eggs singly on submerged macrophytes, usually at the water surface (Cadwallader 1975a; Eldon 1979c; Chapter 9). Eldon (1979c) observed that spawning substrate limitation, as may occur in captivity, can result in eggs being clustered, with these eggs suffering higher rates of mortality, subsequently. Eggs also fall to the sediment, where low dissolved oxygen, proximity to bacteria in the substratum, and smothering, may also lead to high rates of mortality (Eldon 1979c). Thus, the extent of spawning substrate is likely to have consequences for recruitment in *N. burrowsius*. Additionally, *N. burrowsius* prefers to spawn on living vegetation, including algae, rather than artificial substrates, such as tassels of nylon knitting material (Eldon 1979c). This suggests that *N. burrowsius* have particular preferences for the types of substrate used in spawning. Indeed, Eldon (1979c) found many more *N. burrowsius* eggs attached to native macrophytes, e.g. water milfoil (*Myriophyllum* spp.), than introduced species of sweetgrass (*Glyceria* spp.) and Canadian pondweed (*Elodea canadensis*). Yet, in

captivity *N. burrowsius* will also spawn on the glass sides of aquaria (Cadwallader 1975a). Thus, *N. burrowsius* may only be selective when they have the opportunity.

Eldon et al. (1978) suggested that *N. burrowsius* move upstream to suitable spawning locations. Although this has not been subsequently observed, and largely discounted (Eldon 1979c), it is possible that *N. burrowsius* do not spawn randomly, but rather move to particularly suitable habitats. Thus, identification of spawning locations within habitats, and small scale patterns in egg congregations will provide information likely of importance to guide conservation efforts. In particular, the identification of vegetation preferentially used for spawning is essential when making decisions on habitat protection and rehabilitation.

In this chapter I examine the results of a field survey of aquatic flora associated with the occurrence of *N. burrowsius* eggs. Further, outdoor tank experiments were used to determine if the lack of aquatic macrophytes delays spawning and subsequently affects recruitment in *N. burrowsius*.

METHODS

Field survey

To identify spawning locations, a field survey for *N. burrowsius* eggs was initiated after the discovery of eggs at Te Roto Repo o Tawera on 24 August 1999. This initial find was in an area of slow velocity water, with eggs attached to a large patch of macrophytes within a kahikatea (*Dacrycarpus dacrydioides*) stand. The four most important natural habitats of *N. burrowsius* (Eldon 1993; see Chapter 1 for more details) were surveyed within a one week period (27 – 31 August 1999). Collection points within sites were determined using random numbers to generate randomised distances between sample points. Replicate macrophyte samples were taken at each point if macrophytes were abundant. When samples were taken from a discrete macrophyte patch, the length and width of the patch was measured. Each sample location was categorised according to whether it was a macrophyte patch or stream margin vegetation, whether the stream morphology was a pool or a run, and whether the area had contained water during autumn 1999.

Collected samples were examined while plant material was still fresh, with eggs being rinsed from plants using running water over a 0.5 mm mesh. This method, although allowing efficient removal of eggs, resulted in a small loss of plant mass. This unavoidable loss was expected to be similar across samples, and consisted mainly of small floating macrophytes (e.g. duckweed (*Lemna minor*)), algal fragments and detritus, potentially reducing the relative weight of these fractions, thus underestimating their importance. Plant samples were separated into species, dried at 40 °C for 48 hours, with the dry weight of each plant species and the total dry weight of plant material in a sample being recorded.

Tank experiment

To determine whether the absence of macrophytes influenced *N. burrowsius* spawning, egg survival, and recruitment of fry; an experiment was conducted in 2001. Investigations were performed in twelve modified 750 litre cattle troughs situated outdoors. Experimental setup and procedures prior to the experiment were similar to those outlined in Chapter 9. On 14 September 2001 all males had flowing milt and eggs could be easily stripped from several females. Anaesthetised fish were sexed following the guidelines of Cadwallader (1973), as detailed in Chapter 9, and measured, before being randomly allocated to tanks prior to the commencement of the experiment on 17 September 2001. A tank density of six fish (3 male, 3 female) per tank was used. Female length at the start of the experiment was used to calculate an expected fecundity based on Eldon (1979c):

Estimated number of eggs in stage 3 ovaries = $0.0016 \cdot \text{Length (mm TL)}^{3.05}$.

Spawning substrate

Watercress (*Rorippa* spp.) was used as spawning substrate as it is common in *N. burrowsius* habitats. Live *Rorippa* spp., collected from an agricultural ditch where *N. burrowsius* did not occur, was thoroughly rinsed to remove any invertebrates and detritus, before being placed in tanks, where it formed a dense floating substrate with abundant exposed roots (Figure 10.1). The occurrence of spawning was assessed daily with recently spawned eggs being readily differentiated. Egg abundance was measured by counting the number of eggs attached around the rim of the tank and upon the macrophytes. Numbers of eggs on macrophytes were estimated using ten 0.027 m^2 quadrates (Figure 10.1), randomly placed in each tank.

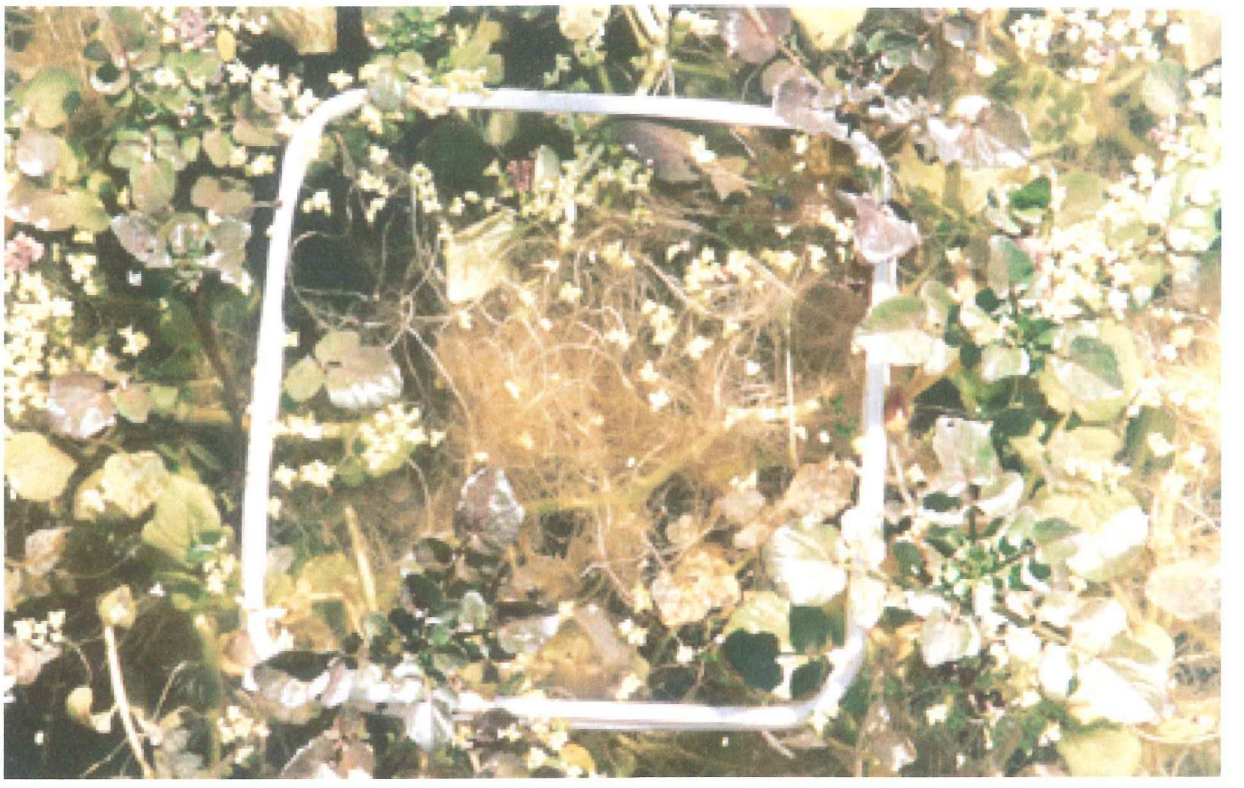

Figure 10.1. Location of a large concentration of *N. burrowsius* eggs amongst watercress roots at the water surface in outdoor tanks. The quadrate was cut from a standard 1 litre ice-cream container lid.

Egg dislodgement

During the experiment, sediment was examined to investigate whether the presence or absence of spawning substrate affected the abundance and state of eggs falling to the sediment. On 11 October 2001, sediment was collected from each tank bottom using a fine mesh hand net (8 x 10 cm), which collected a sample of approximately 300 ml. Three replicate samples were taken from each of the twelve tanks and stored at 4 °C overnight. *N. burrowsius* eggs present were removed from the substratum and examined under magnification against a black background. Transparency of eggs indicated that they were viable, whereas opaque eggs indicated that the embryo had died.

Recruitment to fry

Recruitment of fry was sampled on 27 – 30 October 2001 using 3, 5 minute passes of rigorous sweeps with a fine mesh hand net, in each of the twelve tanks. The total number of fry in tanks was then estimated using the maximum weighted likelihood method of Carle & Strub (1978).

Statistical analysis

All statistical analyses were conducted in Statistica 6.0 (Statsoft Inc. 2001). Kruskal-Wallis tests were used on data which did not fit assumptions of normality. A chi-squared test of independence was used to determine whether eggs were clumped or randomly distributed analyse the distribution of eggs throughout sites. Discriminant functions analysis was used to examine which macrophyte species where associated with the presence of eggs.

RESULTS

Field survey

The abundance of *N. burrowsius* eggs on aquatic vegetation was highly variable (Figure 10.2), with only 21 % of randomly collected macrophyte samples having *N. burrowsius* eggs attached. Further, there was no significant difference in egg density between sites as determined by a Kruskal-Wallis test ($H_{(3, N = 137)} = 7.8$, $p = 0.05$).

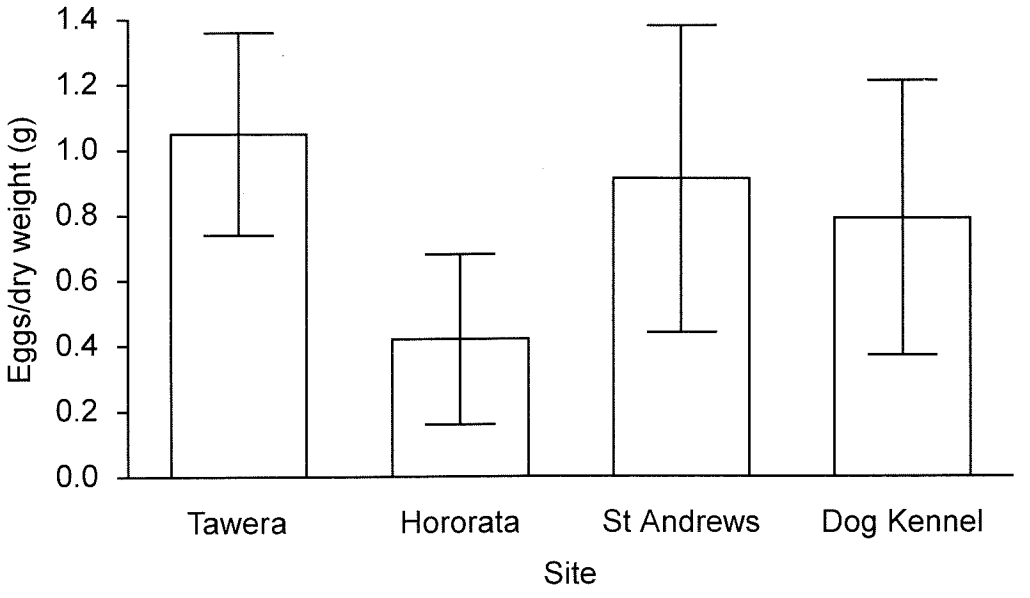

Figure 10.2. Density of eggs collected in August 1999 at each site (mean ± 1 SE), calculated as the number of eggs per total dry weight of aquatic vegetation.

Distribution of eggs

The large variability in egg abundance and the low number of samples with eggs (Figure 10.3) restricted thorough analysis to two sites. Analysis of data collected from Te Roto Repo o Tawera and Hororata Spring found no relationship between the size of a macrophyte patch and the number of eggs found. Eggs were also scattered amongst aquatic vegetation independent of specific location, i.e. there were no significant differences in egg number between the edge and the interior of macrophyte patches, nor between macrophyte patches and stream margin vegetation.

At the intermittently flowing Hororata Spring and Dog Kennel Stream sites, 39 locations were sampled that had dried up, and 31 that had contained water, during the previous autumn. Although the high number of samples with no eggs ruled out statistical analysis, six samples from locations where water had been present contained eggs, whereas only one sample from a location that had dried up did. This may suggest that in intermittent sites, spawning is more likely to occur in areas that dry up less often.

Large numbers of eggs were found at Te Roto Repo o Tawera (Chapter 9) allowing statistical analysis of their spatial distribution. A non-random distribution was found in the number of eggs per dry weight of macrophyte sample per hundred metre reach (χ^2 = 247, df = 11 $p < 0.001$). Areas of egg aggregation were also seen at other sites. For example, egg abundance was high in the Spring Pool at Hororata Spring, and just above, or in the Pool habitat at St Andrews Drain (see Chapter 1 for site descriptions). Thus, although low statistical power prevented thorough analysis into whether *N. burrowsius* were spawning in particular places, results suggest that some level of spawning site selection occurs.

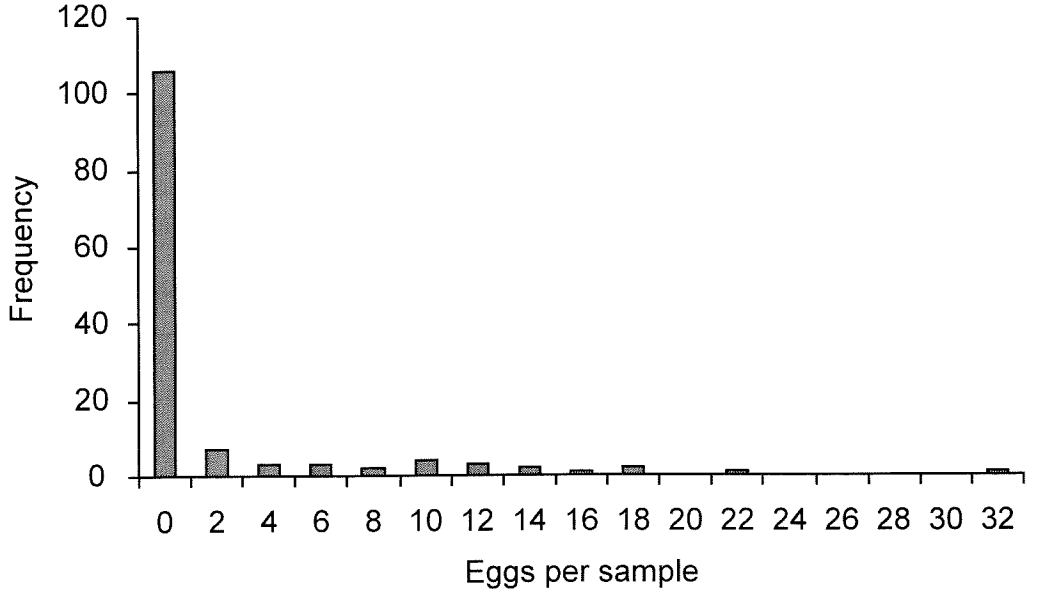

Figure 10.3. Numbers of eggs found in each sample and the frequency of occurrence at the four sites combined.

Egg placement

Eggs, although adhesive, readily detach from the macrophyte substrate when samples are handled (Eldon 1979c). Of 258 eggs collected in total, only 9 % found during sampling were still strongly adhered to macrophytes (Table 10.1), 60 % of which were found on root hairs, 30 % in foliage and 10% on plant stems. These results differ from those of Eldon (1979c), who found eggs predominantly on plant foliage and only 0.4 % of the time on the roots. This difference is most likely due to the structural differences in the macrophyte species examined in the two

studies. The roots of starwort (*Callitriche stagnalis*) and *Rorippa* spp. on which eggs were attached (this study) were adventitious and nearer the water surface, rather than more embedded in the sediment as *Myriophyllum* spp. roots were likely to be (Eldon's study).

Table 10.1. Location and quantity of eggs found still adhered to macrophytes during sampling.

Plant species	Location	Number eggs attached
Filamentous algae	Entangled	13
Callitriche stagnalis	Back of leaf	2
Callitriche stagnalis	Root hairs	3
Rorippa spp.	Stem	1
Rorippa spp.	Back of leaf	1
Rorippa spp.	Root hairs	3

Macrophyte associations

Discriminant function analysis was used to investigate whether particular macrophyte species were more favoured as spawning substrate. Forward stepwise discriminate analysis identified four species of aquatic plant positively associated with the presence of *N. burrowsius* eggs (Table 10.2; Wilks lambda = 0.895, $F_{(2, 131)} = 3.1$, $p = 0.012$, Eigenvalue = 0.12, canonical R = 0.32). All species, except *Myriophyllum propinquum* are common introduced species (Johnson & Brooke 1998). Ubiquitous species such as *Rorippa* spp. were not strong predictors of the presence of *N. burrowsius* eggs, despite finding many eggs attached to these species. A negative association was found between the presence of eggs and dead plant material, which was predominately debris from riparian sources. The classification matrix correctly classified 79 % of samples; moreover 100 % of cases where eggs were absent were correctly identified by this model.

Table 10.2. Standardised coefficients for canonical discriminant function variables for each macrophyte species included in the model, with the level of variance explained in each case. Analysis was conducted on grams dry weight of macrophytes per sample. Dashes indicate species not included in the model. The independent variable in the analysis was the presence or absence of *N. burrowsius* eggs.

Spawning substrate	Coefficient	R^2
Callitriche stagnalis	0.84	0.12
Glyceria declinata	0.74	0.15
Myriophyllum propinquum	0.36	0.06
Glyceria fluitans	0.36	0.06
Dead plant material	-0.3	0.01
Rorippa spp.	-	-
Myosotis laxa	-	-
Lemna minor	-	-
Mimulus guttatus	-	-
Potamogeton cheesemanii	-	-
Ranunculus spp.	-	-
Juncus spp.	-	-
Cyperus spp.	-	-

Tank experiment

Spawning delay

In outdoor tank experiments used to investigate whether the absence of suitable spawning substrate limited the timing and/or success of spawning, it was found that females in tanks with macrophytes present spawned earlier than those in which macrophytes were absent (Figure 10.4). The delay in spawning likely due to the absence of suitable spawning substrate was approximately one week, after which the number of spawned females was similar in both treatments. Although the experimental setup meant that females spawning on the same day in the

same tank could not be differentiated, simultaneous spawning appeared to be an unusual occurrence as spawning by most of the 36 females was accounted for by the appearance of newly spawned eggs.

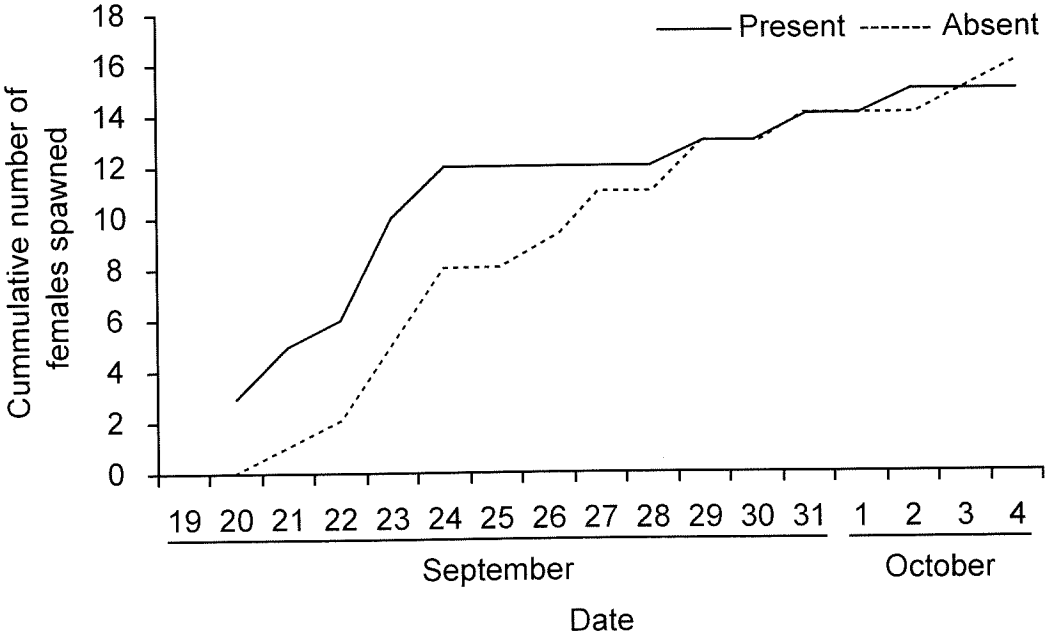

Figure 10.4. Cumulative number of females spawning in tanks with (solid line), and without (dotted line) macrophytes as spawning substrate.

Egg density

The mean number of eggs found in each tank during the experiment was significantly different between those with and without macrophytes (Kruskal-Wallis test: $H_{(1,\ N\ =\ 12)} = 36$, $p = 0.004$). Egg density increased through time and was highest in tanks containing macrophytes (Figure 10.5). High rates of immediate egg loss were witnessed in spawning substrate treatments without macrophytes, especially when eggs were adhered to the sides of the tanks. In one case 238 eggs disappeared overnight from a single tank lacking macrophytes, although, the average rate of egg loss per night was 32 ± 16.6 (mean ± 1 SE).

Mortality during development was readily estimated, with subsequent infections by *Saprolegnia* fungus being common. Overall, observed egg mortality was 16 ± 3.5 % (mean ± 1 SE) across all tanks during the experimental period.

Females appeared to favour certain spawning locations within tanks, particularly two alcoves which were part of the cattle troughs moulded structure, and in the thickest patches of macrophytes, especially the dense floating roots on the sunnier side of tanks.

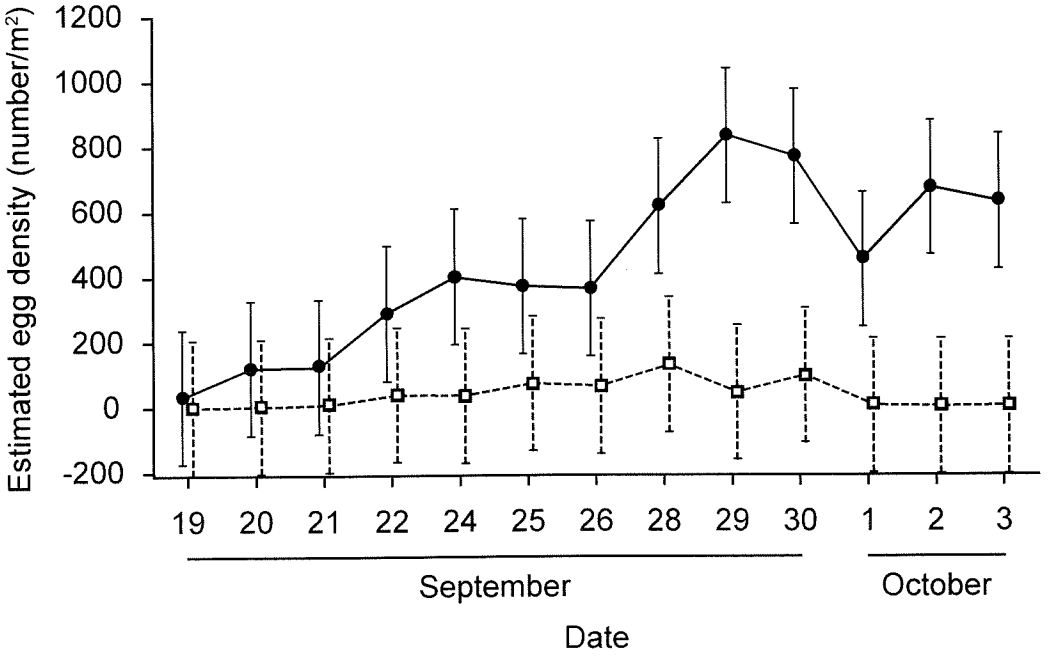

Figure 10.5. Estimated density (mean ± 1 SE) of viable eggs at the water surface in tanks with (solid line) and without (dotted line) macrophytes.

Dislodgement of eggs

It was hypothesised that the lack of spawning substrate would result in increased numbers of eggs falling to the sediment, potentially explaining the low density of eggs found at the water surface and on tank sides in spawning substrate treatments without macrophytes. Overall, 39 eggs were found in the sediment of tanks with, and 70 in tanks without, macrophytes. However, there was no statistical difference in the number of dislodged eggs found in tanks with and without

macrophytes present, as determined by a nonparametric Kruskal-Wallis test ($H_{(1, N = 12)} = 0.1$, $p = 0.7$; Figure 10.6). Overall, mortality of dislodged eggs was 63 ± 6.2 % (mean ± 1 SE).

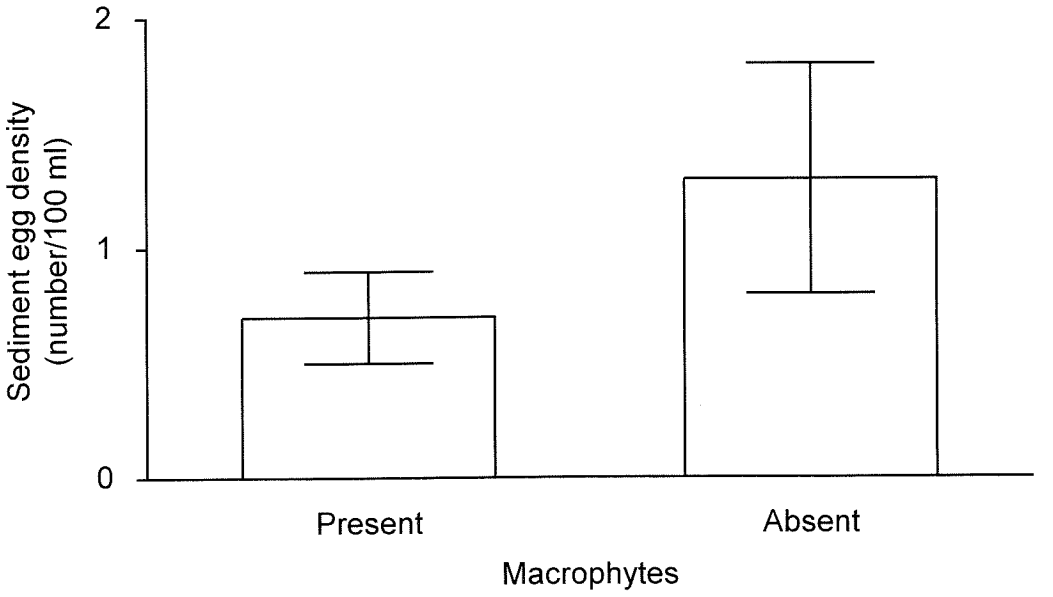

Figure 10.6. Mean density of eggs found on the sediment of experimental tanks (mean number per 100 ml sediment ± 1 SE) in the presence and absence of aquatic macrophytes as spawning substrate.

Recruitment to fry

Eggs were left to hatch and fry develop to a stage readily captured by a hand net. Surprisingly, there was no significant difference between the spawning substrate treatments in the number of fry captured, due to a large unaccounted for variation in fry number between tanks (Figure 10.7). The maximum number of fry found in a single tank was 92, with 5 tanks having no recruitment of fry at all. Four tanks had recruitment of between 10 and 18 fry. Plotting the estimated fecundity, abundance of eggs and subsequent number of fry which survived showed that in many cases, tanks with macrophytes had the greatest decline in numbers between the egg and fry stages (Figure 10.8).

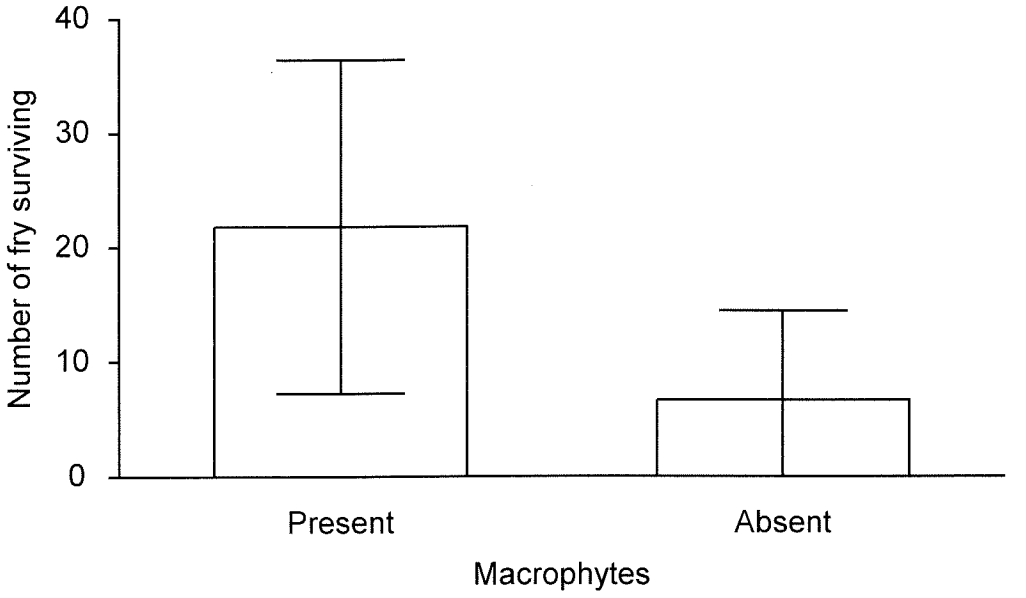

Figure 10.7. Recruitment of fry in tanks with and without macrophytes present (mean estimated number of fry ± 1 SE).

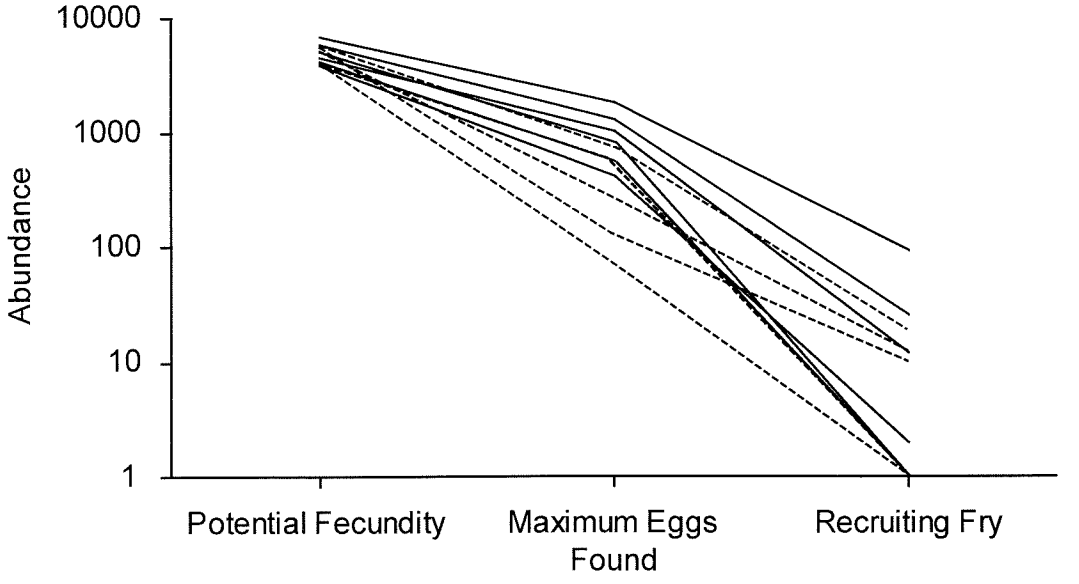

Figure 10.8. Abundance of developmental stages in each of twelve experimental tanks with (solid line), and without (dotted line), macrophytes present. Comparison of the potential fecundity of females present (based on stage 3 ovaries as described by Eldon (1979c)) was calculated from the size of females in tanks; estimated maximum number of eggs found during the experiment, and the number of fry subsequently captured. Note the log scale of y-axis.

DISCUSSION

This study demonstrated that spawning in *N. burrowsius* was delayed in the absence of macrophytes, indicating that the availability of suitable substrate may limit successful spawning. Further, particular macrophyte species were associated with the presence of eggs in the field. These macrophyte species typically provided a complex architecture at the water surface, either by root hairs, e.g. *Glyceria* spp., or through leaf structure, e.g. *Myriophyllum* spp. Thus, certain macrophyte species, via their growth form may play an important role as spawning substrate for *N. burrowsius*. Furthermore, the microhabitat preferred by adults appears different from that used for spawning. For example, *C. stagnalis* was negatively associated with the presence of *N. burrowsius* adults (Chapter 3), yet positively associated with the presence of eggs. Further, *Glyceria* spp., despite not being associated with the presence of adult *N. burrowsius* (Chapter 3), were strongly associated with the presence of eggs in samples. As *C. stagnalis* and floating sweetgrass (*Glyceria fluitans*) are often abundant in shallower backwater areas and pool margins, adults may move into such areas to spawn. This may be a mechanism to reduce cannibalism and/or improve survival of early life stages.

Macrophytes may provide a complex substrate at the oxygen rich water surface, cover for adults during the conspicuous spawning period, and camouflage for vulnerable eggs (Figure 10.9). However, macrophyte cover fluctuates seasonally, being substantially lower in winter, particularly after removal by high flows (Howard-Williams et al. 1982; Riis et al. 2003). As *N. burrowsius* spawns in late winter and early spring it is possible that after exceptional winter flooding, macrophyte densities may be low. Thus, delaying spawning until macrophytes have had time to re-establish may be a common response in *N. burrowsius*. Furthermore, *N. burrowsius* appear to readily delay spawning in response to habitat conditions, which is likely to optimise progeny survival (Chapter 9). The importance of suitable spawning substrate has also been identified in related Galaxiidae. For instance, it has been suggested that lack of suitable spawning substrate may cause Canterbury (*Galaxias vulgaris*), and roundhead galaxias (*G. anomalus*), to reabsorb eggs rather than spawn in unsuitable conditions (Moore et al. 1999).

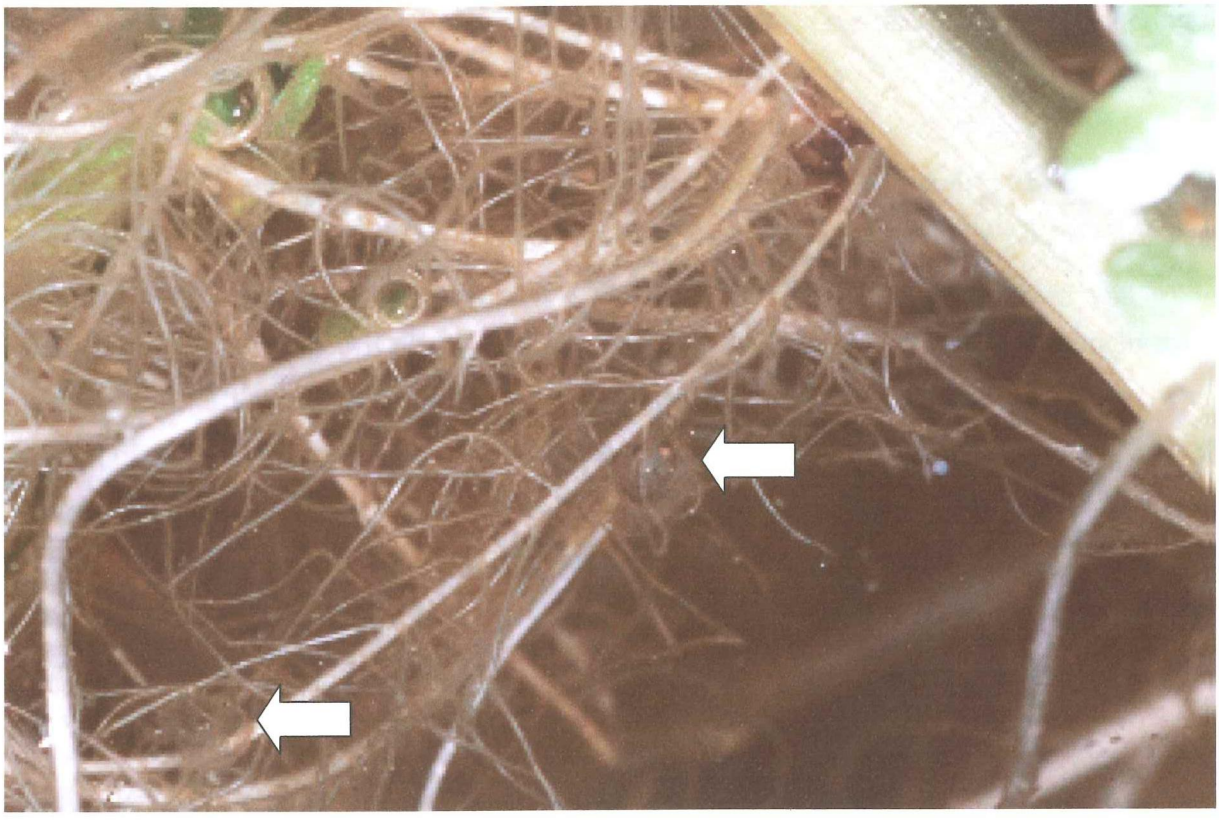

Figure 10.9. Aquatic vegetation camouflages *N. burrowsius* eggs (indicated by arrows) attached to exposed root hairs of floating *Rorippa* spp. Eggs are often difficult to differentiate from gas bubbles emitted by plants (top left). Photo by A. McIntosh.

N. burrowsius frequently occurs in habitat prone to hypoxia (Eldon 1979a). Although embryos do have a limited ability to compensate for low dissolved oxygen (Chapter 11), high mortality was observed in eggs that had fallen to the sediment. Thus, it may be important that eggs are deposited at or very near the water surface. Cleaned *N. burrowsius* eggs are very adhesive, enabling them to 'stick' to the water meniscus despite being slightly negatively buoyant (personal observation, see Chapter 11 for cleaning method). Most freshwater fish species do not have neutrally buoyant eggs, although some are almost so, such as those of the striped bass (*Morone saxatilis*) in which buoyancy is achieved through the presence of a large oil globule (Mangor-Jensen et al. 1993). Furthermore, Davidson (1949) stated that brown mudfish (*N. apoda*) eggs were more buoyant than those of inanga (*Galaxias maculatus*). Oil globules are present in the eggs of *Neochanna* (Perrie 2004; personal observation), and in the eggs of *Galaxias* species such as *G. maculatus*, which position their eggs in vegetation along the bank edge (Benzie 1968), and banded kokopu (*G. fasciatus*), another terrestrial spawner (Hopkins 1979). Thus, extremely

adhesive eggs with a slight negative buoyancy are likely to improve their chances of adhering to substrate near the water surface.

The nature of a macrophyte, in particular its structural complexity is likely to be important in ensuring egg adherence. As noted, macrophyte species associated with *N. burrowsius* eggs tended to have complex architecture; increasing the chance that a slowly sinking egg would encounter vegetation. However, other factors may also be important, such as the roughness of plant surfaces. This study only found eggs still attached to *C. stagnalis* and *Rorippa* spp., while Eldon (1979c) found eggs remained attached to *Myriophyllum* spp. more readily than *E. canadensis* and *Glyceria* spp. Thus, *N. burrowsius* eggs could be more readily dislodged from certain macrophyte species, although the mechanisms behind such observations are unclear. However, in the field, eggs are likely to be dislodged by disturbances from flooding, stock and waterfowl.

The fate of eggs

Only a small fraction of the number of eggs estimated from female sizes were accounted for in tanks, even when macrophytes were present. Differences between estimated fecundity and subsequent egg density and survival could have several explanations. These include, failure of females to fully spawn, failure of males to completely fertilise eggs, poor placement of eggs leading to them falling to the substratum, problems in embryo development, predation by invertebrates and cannibalism. Determining the relative importance of these factors is difficult, but the factor(s) would have to be capable of causing mortality in thousands of eggs. The large number of 'missing' eggs is unlikely to be explained by a failure of females to fully spawn. Allibone & Townsend (1997b) in a survey of the reproductive biology of four non-migratory *Galaxias* species, found partially spent females during the spawning period, however each of these contained fewer than 20 eggs. Mortality of spawned *N. burrowsius* eggs was observed, the most likely cause being a combination of fungal infection, developmental flaws, and failure to be fertilised. However, the level of such mortality does not explain the number of missing eggs.

The tanks were intended to be representative of natural habitats, so they contained a diverse range of macro-invertebrates. Potentially predatory species of flatworms, caddisfly larvae, and Odonata larvae were all present. However, no congregations of carnivorous invertebrates were

observed near eggs. In a comprehensive study of lentic macro-invertebrate consumption of perch (*Perca fluviatilis*) eggs, two species of flatworms and three species of caddisfly were found to consume eggs (Diamond & Wakefield 1986). Further, invertebrate predation was found to be an important source of mortality on rainbow trout (*Oncorhynchus mykiss*) eggs by Brown & Diamond (1984). Importantly, however, invertebrates are seldom able to consume the entire egg and often only leave small puncture wounds (Fox 1978), which likely leads to subsequent infection. Thus, mortality due to invertebrate predation is likely to have been included in dead egg counts and so is an unlikely cause of egg disappearance.

The most compelling cause for the dramatic difference between potential fecundity and subsequent egg counts is cannibalism. Behaviour observed during spawning suggests immediate acts of cannibalism. During the act of spawning, fish were observed to follow behind the female and her partner and it appeared that they were eating eggs as they were spawned, evidenced by regular gulping movements. It is unlikely that this gulping behaviour was directly related to mating. Moreover, Perrie (2004) observed male black mudfish (*Neochanna diversus*) consuming eggs during the act of spawning. My study also indicates that cannibalism is higher if camouflaging aquatic vegetation is scarce, as egg loss was greatest in spawning substrate treatments without macrophytes. Furthermore, substantial numbers of eggs disappeared over night subsequent to spawning. Cannibalism has been previously recorded in *N. burrowsius*; with both investigations of *N. burrowsius* diet having found evidence of egg cannibalism (Cadwallader 1975a; Eldon 1979c), even though these studies were not specifically conducted during the peak spawning period. Cadwallader (1975a) found that *N. burrowsius* eggs were the third most abundant item eaten. Adults had eaten conspecific eggs at three locations and at one site a total of 23 eggs were found in the gut contents of thirteen fish. In a larger study, 1.7 % of *N. burrowsius* had cannibalised eggs, even though samples were taken over a nine month period (Eldon 1979c). Thus, cannibalism could be a major source of *N. burrowsius* egg mortality, and is likely to have important ecological implications.

Cannibalism is common in most teleost families (Kume et al. 2002), and can be substantial. For example, Vik et al. (2001) found that 20 % of adult brown trout (*Salmo trutta*) had cannibalised parr. Further, cannibalism of eggs has been reported in several species of Galaxiidae (Eldon et al. 1978; Kusabs & Swales 1991; Allibone & Townsend 1998). Moreover, the risk of egg predation by fish has been implicated as the mechanism explaining the common occurrence of terrestrial

spawning (Eldon 1971; Allibone 2003; Charteris et al. 2003). Few New Zealand studies have directly investigated egg cannibalism, however Allibone (2003) found that 39 out of 45 *G. maculatus* examined had recently cannibalised eggs, and from the 335 adults captured in the spawning grounds he estimated that 5382 eggs could have been consumed.

Cannibalism may benefit *N. burrowsius* males who were unable to mate with a female, by direct energy acquisition and reduction of a competitor's genetic input into the population. Evidence suggests that female *N. burrowsius* spawn independently, thus there are likely to be more males than females attempting to spawn at any time. Furthermore, Eldon (1979c) found males to be more active during the spawning period than at other times. Thus, cannibalism is likely to be beneficial for males, yet detrimental to females. The production of 'dummy eggs', lacking a yolk, has been observed in cardinalfish (*Apogon lineatus*), a species in which the mouth brooding male habitually cannibalises its progeny (Kume et al. 2002). Several unusual smaller *N. burrowsius* eggs, which appeared to lack embryos, were observed. However the importance of these was not realised at the time and further investigation was not carried out. However, if cannibalism by males is widespread, females may dilute the risk to their fitness by unevenly investing in eggs. Further study into the consequences of cannibalism in *N. burrowsius* is required as it is possible that cannibalism affects population regulation.

The fate of fry

Surprisingly, despite the number of eggs estimated to occur in tanks with macrophytes being an order of magnitude greater than in those without macrophytes, there was no significant difference between spawning substrate treatments in recruitment to the fry stage. The greater proportion of eggs surviving till fry stage in the absence of macrophytes indicates that additional factors control recruitment of fry. This result emphasises the importance of considering each life stage, as they usually require differing conditions, especially in species which undergo an ontological shift in habitat, such as *N. burrowsius*. While aquatic vegetation is utilised for spawning, pelagic fry of *N. burrowsius* tend to congregate in backwater areas free of macrophytes (personal observation). This is likely related to ease of fry movement as they struggled to swim in dense macrophytes, and could be related to food resources, such as zooplankton (Burks et al. 2002).

In this study the spawning substrate treatment tanks containing macrophytes were covered with a dense surface layer of vegetation. This may not only have interfered with foraging but also put fry within proximity of invertebrate predators such as damselflies (Odonata). Damselflies were abundant in the macrophyte containing tanks, as they also require aquatic vegetation for oviposition. Laboratory experiments indicate that damselflies are capable of consuming nearly 4 *N. burrowsius* fry per 24 hour period (O'Brien 2000). Although some damselflies actively stalk prey, most rely on ambushing passing fry. Damselflies are 'wasteful consumers' often leaving much of the fry body unconsumed, as they are unable to manipulate prey with their mouthparts (personal observation). Thus, damselflies have the capacity to predate on large numbers of *N. burrowsius* fry before satiation. Invertebrate predation is therefore likely to explain the reduced recruitment of fry in spawning substrate treatment tanks containing macrophytes. However, cannibalism cannot be discounted, although, temporal segregation between diurnal fry and nocturnal adults may limit this. Thus, although low macrophyte abundance may affect spawning and egg survival, dense macrophyte beds may increase fry mortality. The low recruitment, cannibalism and predation recorded in my experiments could have been exacerbated by experimental tank conditions, such as water quality, food limitation, spatial confinement and lack of habitat heterogeneity. Nonetheless, in wild populations, very low recruitment was found in sites with large adult *N. burrowsius* populations (Chapter 3).

Overall macrophytes play an important role in the recruitment success of *N. burrowsius*. The presence of macrophytes reduces the number of eggs falling to the sediment, and the level of cannibalism, both of which appear to be significant causes of *N. burrowsius* egg mortality. However, this study also illustrates the likely importance of habitat heterogeneity in preventing predation early in the life history of *N. burrowsius*. Conservation measures which encourage particular macrophyte species, and a range of open and vegetated patches, especially in backwaters, are likely to promote recruitment in *N. burrowsius*.

Chapter 11.

Mechanisms moderating acute and chronic exposure to hypoxia in *Neochanna burrowsius* embryos.

INTRODUCTION

Oxygen is a fundamental requirement for respiration in vertebrates. Its ubiquitous abundance, as a component of air, reduces in water due to its low dissolvability, which is inversely related to temperature. In lentic habitats and slow-flowing streams, wide fluctuations in dissolved oxygen levels, especially within dense macrophyte patches, can occur diurnally (Wilcock et al. 1998). Consequently, dissolved oxygen levels can have a strong selective influence on the physiology and behaviour of aquatic species (Brungs 1971). Many fish species inhabiting periodically hypoxic environments have evolved adaptations such as a low metabolic rate, and tolerance of low oxygen tensions; or can supplement oxygen requirements by breathing air with modified swim bladders (Chapman et al. 2002a). Most adaptations are restricted to post embryonic stages, where physiological development is complete. In contrast, eggs are vulnerable to hypoxia; in that being sessile they are largely incapable of avoiding adverse environmental conditions (Blaxter 1969). To overcome this, some early egg development in salmonids can continue in anoxic conditions, possibly due to oxygen stored within the perivitelline fluid of the egg (Blaxter 1969). However, as embryos develop oxygen requirements increase and the ability to survive low dissolved oxygen levels declines.

The focus of this study, Canterbury mudfish (*Neochanna burrowsius*), occurs in remnant wetlands which are frequently hypoxic during summer and autumn (Eldon 1979a). Adults exhibit a variety of physiological adaptations, including high cutaneous gaseous exchange, a low metabolic rate and a tolerance of fluctuating internal and environmental conditions (Meredith 1985). Furthermore, *N. burrowsius* exhibit behavioural responses such as air breathing and actively leaving hypoxic waters, which considerably enhance their survival ability (Meredith 1981). Thus, adults may largely avoid hypoxic events, by finding refuge in moist terrestrial

locations where they can remain emersed for considerable periods (Eldon 1979c). For eggs, contrastly, avoidance of hypoxic water is a matter of chance and timing. Adult *N. burrowsius* scatter their eggs close to the water surface, where oxygen levels are greatest. However, *N. burrowsius* show no other parental care. In comparison, upland bully (*Gobiomorphus breviceps*), which frequently co-occurs with *N. burrowsius* (Eldon 1979a; Chapter 8), exhibits parental care, including the fanning of eggs to improve water flow and oxygenation (McDowall 1990). Usually *N. burrowsius* eggs develop in normoxic conditions as *N. burrowsius* begin spawning in late-winter when water levels are high and temperatures low. However, in some intermittent habitats there is only a short period of opportunity for egg and fry development before hypoxic summer conditions (Eldon 1979c). Eldon (1979c) found higher rates of mortality and deformity of *N. burrowsius* embryos at higher temperatures, and suggesting reduced dissolved oxygen as a contributing factor. Further, low dissolved oxygen levels during spawning, and early developmental stages have been implicated as a factor resulting in population extinction (Eldon 1993). Thus, determining the degree of oxygen saturation required for successful recruitment is crucial (Eldon 1993; Bohlen 2003).

In the previous two chapters I detailed situations where *N. burrowsius* delayed spawning. Delays in spawning will increase the likelihood that eggs are exposed to high temperatures and reduced dissolved oxygen. In this chapter I examine the influence of acute and chronic exposure to conditions likely to be encountered if spawning is delayed. Two laboratory investigations were conducted. The first involved measurement of oxygen consumption of eggs by exposing eggs to extremely low dissolved oxygen levels for a short duration. The second experiment involved exposing eggs to elevated temperatures and partially reduced dissolved oxygen levels for the duration of development. Thus, in total these experiments investigated the ability of *N. burrowsius* eggs to tolerate acute periods of low dissolved oxygen, and the consequences of chronic exposure to low dissolved oxygen on subsequent development.

METHODS

Oxygen consumption

Laboratory experiments were conducted to investigate oxygen consumption and the extent that acute exposure to hypoxia influenced *N. burrowsius* egg development. A closed box respirometer cell was used which contained 1.13 ml of water maintained at 15 °C. Prior to use the cell was sterilised for 2 hours with sodium hypochlorite, soaked overnight with distilled water, and thoroughly rinsed. *N. burrowsius* eggs were also cleaned as they readily accumulated detritus due to their adhesive nature. Microscopic nematodes on egg surfaces were removed with forceps, with the aid of a microscope. To remove additional detritus, eggs were stirred with a magnetic flea in distilled water that was exchanged three times. Cleaned eggs were extremely sticky and near neutrally buoyant.

Oxygen consumption ($\dot{M}O_2$) of eggs was measured as the rate of decrease in the oxygen partial pressure of water (PO_2) in the closed cell. An advantage of this method was that the egg's own metabolism generated progressive hypoxia in the chamber. Eggs from the same spawning event of a captive *N. burrowsius* population were collected and kept at 15 °C. Measurements were conducted on stage I and II eggs (see Appendix 1 for details of egg stages used in this thesis). Subsequent to determining oxygen consumption, eggs were maintained at 15 °C until they hatched, to investigate the effects of acute hypoxia exposure on embryo development and survival. Patterns in oxygen consumption were analysed to identify PO_2 critical ($PO_{2\ crit}$) values, i.e. the change from oxygen-regulating to oxygen-conforming patterns. Oxygen conformity describes the situation where oxygen consumption declines proportionately to the oxygen partial pressure of water, while oxygen regulation is the ability to maintain oxygen consumption despite a declining partial pressure of oxygen in water. Curve inflection points indicating $PO_{2\ crit}$ values where oxygen consumption changed, were calculated in Excel 10 (Microsoft Corporation 2002), by separating the curve into separate series and fitting extrapolated linear trend lines to each section of curve. Linear regression and homogeneity of slopes tests were conducted in Prism 3.0 (GraphPad Software 1999), to analyse the slopes of curves during oxygen regulation.

Chronic exposure

To investigate the effect of chronic exposure to moderately reduced dissolved oxygen levels at elevated temperatures, timing of hatching and embryo development were analysed using a factorial experimental design. The two factorial treatments involved the manipulation of dissolved oxygen levels, and placement relative to the water surface. Eggs were placed either just below the water surface or at the bottom of experimental containers. This was to investigate the consequences to eggs of being dislodged from macrophytes and falling to the sediment (Chapter 10). In this experiment dissolved oxygen levels were lowered by heating water to 60 °C, thus removing oxygen due to its decreased solubility at higher temperatures. Treated water was then placed in 12 two litre containers, and left undisturbed to cool to room temperature (20 ± 2 °C). Once cooled, six containers were then re-oxygenated using air bubbled through an air stone. This process achieved a significant difference in dissolved oxygen saturation (Figure 11.1). Dissolved oxygen levels relative to air saturation were 80 % in low oxygen treatments and 95 % of air saturation in high oxygen saturation treatments. Further to the variations in dissolved oxygen between oxygen treatments, it is likely that there were differences in dissolved oxygen levels between water at the surface and bottom of individual containers.

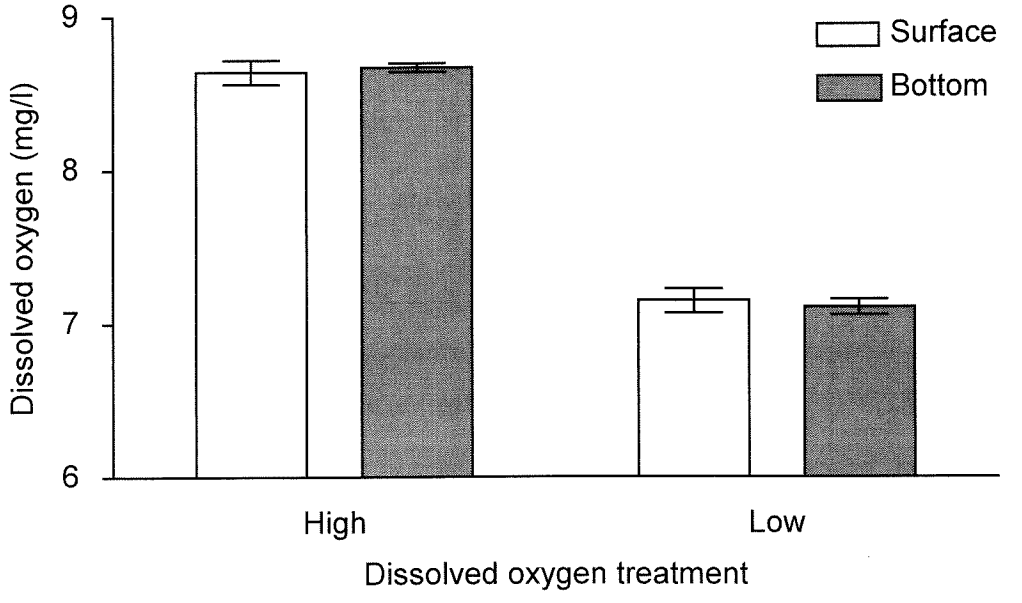

Figure 11.1. Dissolved oxygen levels (mean ± 1 SE) of water in high and low dissolved oxygen treatments at 19.8 °C used in the chronic exposure experiment.

Newly spawned *N. burrowsius* eggs were collected on 10 October 2000 from a captive population and acclimated at 4.5 °C for nine days, then at 15 °C for 24 hours. Experiments commenced on 20 October 2000, with eight eggs being placed on a mesh platform attached to the bottom of each of twelve small plastic cups. Each of the twelve containers contained an air stone, although only high oxygen saturation treatment containers received aeration, which was controlled to reduce turbulence. Small fishing-line weights were used to secure the plastic cup in either of two positions as detailed in Figure 11.2. Placement on these cups facilitated exposure of eggs to water at either the surface or bottom of the container.

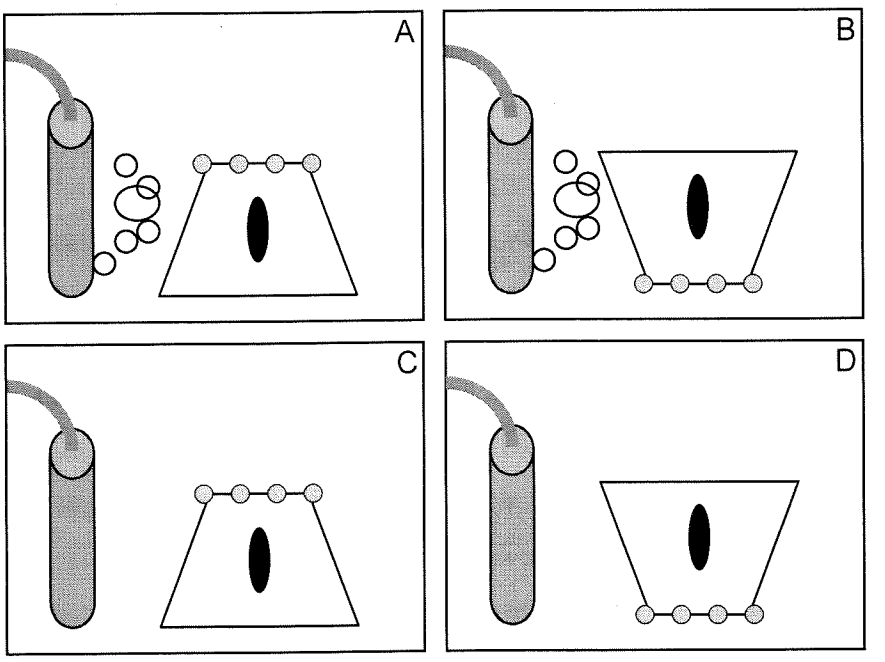

Figure 11.2. Factorial experimental design used to investigate development and hatching of *N. burrowsius* eggs. The high oxygen saturation treatment involved bubbling air through airstones (A and B), whereas low oxygen treats did not (C and D). In the egg position treatment eggs were either placed near the water surface (A and C), or on the bottom of the container (B and D). Cups (parallelogram outline) acted as pedestals with mesh on their smallest side to support eggs (grey circles). Each container contained an air stone (grey cylinder), a weight (black oval) and a plastic cup.

Statistical analysis of this experiment used factorial ANOVA in Statistica 6.0 (StatSoft Inc. 2001), on development time. Differences in developmental times between treatments were tested by ranking hatching time amongst all treatments. This dependent variable was normally distributed (Shapiro-Wilks normality test: $W = 0.95$, $p = 0.31$). Developmental success was tested with dependent variables being the number of eggs hatched alive (log_{10} transformed) and the proportion of fry that hatched that were deformed (arcsine square root transformed). Independent variables were dissolved oxygen levels (high and low) and position of eggs in containers (surface and bottom).

RESULTS

Acute exposure

Oxygen consumption of eggs

Groups of 10 and 12 *N. burrowsius* eggs took approximately seven hours to completely utilise the dissolved oxygen in 1.13 ml of initially oxygen-saturated water (Figure 11.3). Initial oxygen consumption was higher in the more developed stage II eggs. Moreover, there was an intriguing pattern observed in the oxygen consumption of *N. burrowsius* eggs at both stages, although this was most pronounced in stage II eggs (Figure 11.4). It was found that the declining partial pressure of oxygen appeared to trigger a rapid response in embryos, whereby oxygen consumption was reduced and apparently regulated for a period until oxygen levels were lowered to a critical point after which oxygen conformity ensued (Figure 11.4). Thus, oxygen consumption curves for *N. burrowsius* eggs had two inflection points, indicating upper and lower critical values (Table 11.1). This differs from standard oxygen consumption patterns in which organisms do not initially respond to dropping oxygen partial pressure; instead maintain oxygen consumption until a final $PO_{2\ crit}$ value is reached (Blaxter 1969).

The slope of oxygen consumption curves during oxygen regulation was significantly different between stage I and II eggs (homogeneity of slopes test: $F_{(1, 13)} = 6.1$, $p = 0.028$). Stage I eggs had the greatest capacity to regulate consumption as the slope ($b = 0.0012 \pm 0.002$) between $PO_{2\ crit}$ values was not significantly different from zero (linear regression: $F_{(1, 2)} = 0.3$, $p = 0.6$). Thus, stage I *N. burrowsius* eggs were able to maintain a stable oxygen consumption of 3.5 ±

0.218 mmol/egg/h, despite dissolved oxygen levels falling from 82 to 22 % of air saturation. Stage II eggs showed a lower ability to regulate oxygen consumption, with the slope ($b = 0.021 \pm 0.005$) between $PO_{2\ crit}$ values being significantly different from zero (linear regression: $F_{(1, 11)} = 20.93$, $p < 0.001$).

The upper $PO_{2\ crit}$ value was similar for both stage I and II eggs, with both developmental stages rapidly lowering oxygen consumption until dissolved oxygen levels were approximately 80 % of air saturation (Table 11.1). However, the lower $PO_{2\ crit}$ value was different between the stages, with stage I eggs being able to maintain oxygen regulation at a lower dissolved oxygen level.

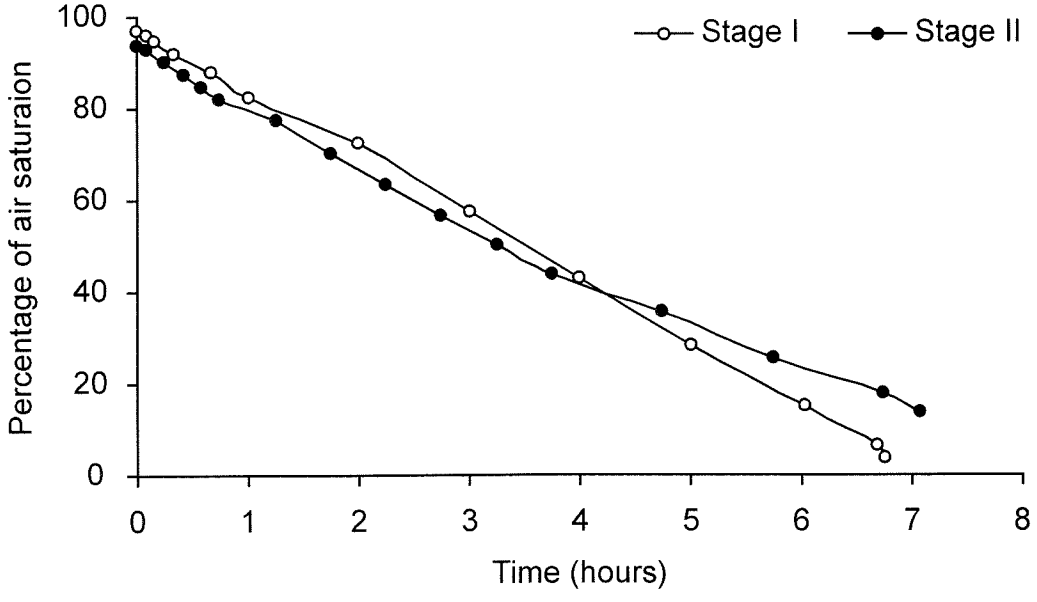

Figure 11.3. Time taken for *N. burrowsius* eggs to reduce dissolved oxygen levels, via oxygen consumption, in a closed cell respirometer. Combined results are shown for 12 Stage I and 10 stage II eggs. Each point indicates an oxygen reading.

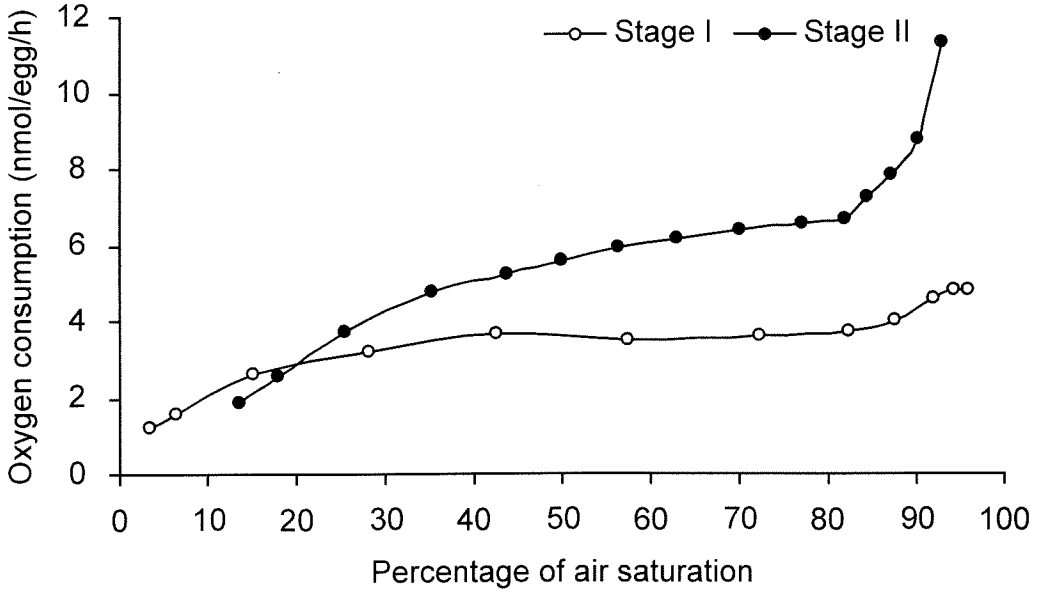

Figure 11.4. Aerobic metabolic rate of *N. burrowsius* eggs with decreasing oxygen partial pressure. Rate of oxygen uptake ($\dot{M}O_2$) is plotted against mean cell partial pressure of oxygen (PO_2), during conditions of increasing hypoxia in a closed cell respirometer. Open and closed circles represent results from stage I and II eggs, respectively.

Table 11.1. Critical values (at 15 °C) in the rate of decrease in the partial pressure of oxygen (PO_2) indicated by inflection points of oxygen consumption curves (Figure 11.3). Critical values ($PO_{2\ crit}$) for dissolved oxygen are given as the percentage of air saturation and as oxygen tension (unit = torr (mmHg)).

Egg stage	Upper critical value		Lower critical value	
	% air saturation	Oxygen tension	% air saturation	Oxygen tension
Stage I	82	128	22	35
Stage II	84	130	32	50

Subsequent development of eggs

N. burrowsius eggs were capable of surviving short periods of extremely low dissolved oxygen. This was demonstrated by the subsequent successful hatching of eggs that had been experimentally exposed to dissolved oxygen levels of circa 10 % of air saturation. However, the timing of exposure may be important as although all embryos that were exposed to hypoxia during stage I hatched, only seven survived of those exposed at development stage II.

Chronic exposure

Hatching experiments examined developmental success and hatching timing of eggs kept at elevated temperatures and at reduced dissolved oxygen levels. A five day delay in the start of hatching was seen in eggs held at 80 % oxygen saturation, indicating depressed metabolic function (Figure 11.5). Thus, dissolved oxygen level had a significant effect on developmental time of eggs (ANOVA: $F_{(1, 8)} = 5.86$, $p = 0.04$, $R^2 = 0.73$). There was no significant effect on position on developmental time.

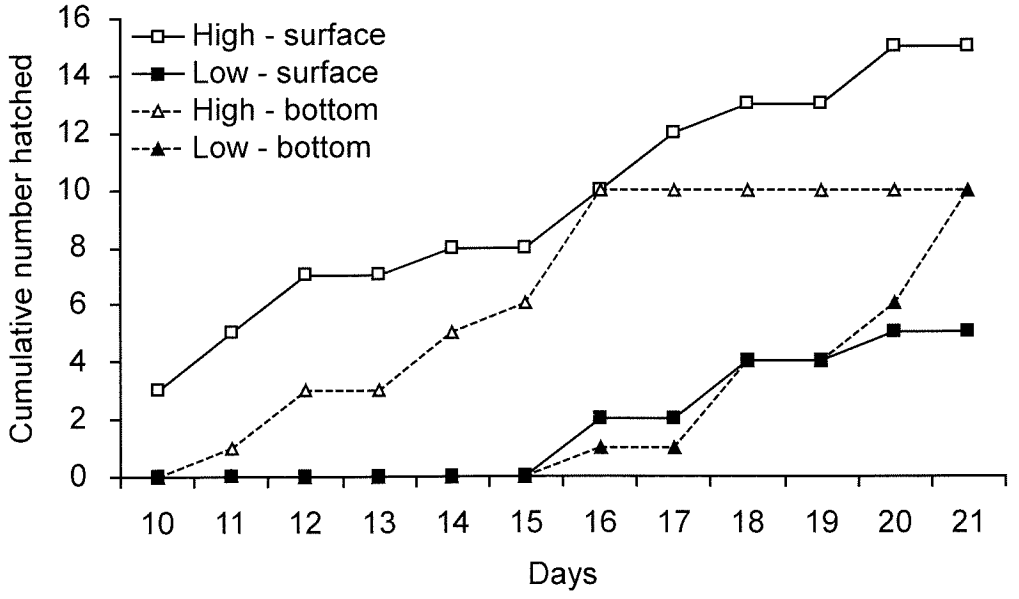

Figure 11.5. Cumulative number of eggs hatched per day since date of spawning in the chronic exposure experiment. Eggs developed at differing dissolved oxygen levels (high and low) and in differing positions within experimental containers (surface and bottom).

There was no significant difference in the number of embryos that hatched alive between treatments. However, factorial ANOVA analysis of the proportion of fry that hatched in deformed states indicated a significant interaction term between oxygen level and egg placement (Table 11.2, Figure 11.6). This was due to low deformity rates of eggs placed nearer the surface of water with higher dissolved oxygen levels. The most prevalent visually-assessed deformity was a curvature of the spine, with fry retaining an embryonic foetal position. These fry were able to actively swim, but only in small circles. It appeared that the observed deformities resulted from premature hatching of the fry, rather than a developmental abnormality; however evaluation of the long-term survival of these fry was not conducted.

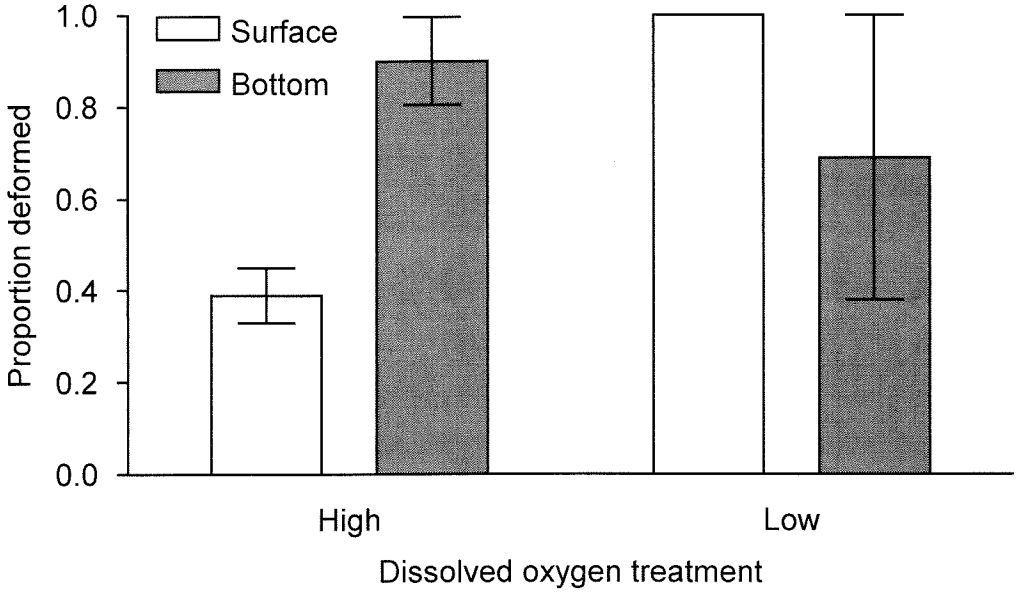

Figure 11.6. Proportion of hatched fry (mean ± 1 SE) which showed obvious signs of deformity in each dissolved oxygen treatment, with eggs placed either at the water surface or on the container bottom in the chronic exposure experiment.

Table 11.2. Results of factorial ANOVA investigating the influence of dissolved oxygen level (O_2) and position of eggs in container (Position) on the proportion of alive, but deformed *N. burrowsius* fry in the chronic exposure experiment.

Source	SS	df	F-ratio	*p*-value
O_2	0.10	1	2.7	0.14
Position	0.03	1	0.7	0.42
O_2 x Position	0.46	1	11.9	0.01*
Error	0.27	7		

DISCUSSION

This study demonstrated that *N. burrowsius* embryos were capable of surviving brief acute hypoxia, and appeared to exhibit physiological responses likely to enhance survival in situations of periodic low dissolved oxygen. Nevertheless, if spawning is delayed, eggs may also experience elevated water temperatures, which would further decrease dissolved oxygen concentrations and potentially lead to chronic hypoxic conditions. However, *N. burrowsius* usually scatter their eggs very close to the water surface where oxygen levels are generally higher (Cadwallader 1975a; Eldon 1979c). This study showed the benefit of this behaviour because even at the elevated temperatures used in this study, eggs placed at the water surface developed quickly and hatched with low levels of deformity.

Early embryonic stages can tolerate lower partial pressures of oxygen than latter stages, as their metabolic needs are less, thus the older stages intolerance levels are lower (Walsh et al. 1989). This was observed in *N. burrowsius* eggs, where oxygen consumption in stage II eggs was nearly twice that of stage I eggs. In Atlantic cod (*Gadus morhua*), an almost tenfold increase in oxygen uptake was measured over the entire development from unfertilised eggs to those immediately prior to hatching (Davenport & Lönning 1980). With increasing oxygen consumption there was also a reduced ability of *N. burrowsius* eggs to compensate for declining partial pressures of oxygen. Further, the lower critical oxygen tension for each stage was different, with stage I eggs

able to regulate oxygen uptake at lower oxygen tensions. Thus, exposure to reduced dissolved oxygen will have a greater adverse effect on latter developmental stages, a period when oxygen supply has greatest importance (Viljanen & Koho 1991).

The response of *N. burrowsius* eggs to increasing hypoxia exhibited three stages, an initial rapid decrease in oxygen consumption, followed by a period of oxygen regulation, and then an eventual limit of regulatory ability was reached with oxygen uptake declining as eggs became stressed. The initial drop in oxygen uptake suggests a physiological response to a decreasing gradient in the partial pressure of oxygen. This ability is unusual, as eggs and adult organisms usually respond to declining oxygen tensions by maintaining oxygen regulation until their tolerance is surpassed ($PO_{2\ crit}$ point), after which their oxygen consumption declines (Blaxter 1969; Grigg 1969). The unusual response observed in *N. burrowsius* eggs could indicate that embryos enter a state of quiescence, which is likely to be an adaptation to frequent short-term hypoxic events. Quiescence is a state of developmental arrest and metabolic depression induced directly by unfavourable environmental conditions (Podrabsky & Hand 1999). In teleosts the ability to reduce oxygen consumption and metabolic rate during embryonic stages is most pronounced in annual species of killifish and rivulines (Cyprinodontiformes; Hand & Podrabsky 2000). These small fish inhabit ephemeral pools in savannah and desert regions of South America and Africa which dry up seasonally, killing adult fish. However, populations are able to persist due to the occurrence of eggs capable of entering a state of diapause. Diapause is similar to quiescence, involving a depression of metabolic rate; however it is initiated without exogenous cues. Incredibly, embryos of the annual rivuline species *Austrofundulus limnaeus*, during one of its three diapause states, has a rate of oxygen consumption 90 % less than during normal development (Podrabsky & Hand 1999). Considering that such ability is not unique, it is possible that the initial drop in oxygen consumption in *N. burrowsius* is an adaptive response to hypoxia which may involve a quiescent state, which would conserve oxygen. However, further detailed research would be required to ascertain the validity of this conclusion.

The critical oxygen point values determined for Stage I *N. burrowsius* eggs were less than those of adults, which have a critical point of 50 – 60 torr (Meredith 1981). Critical oxygen tension values ($PO_{2\ crit}$), although a valuable tool in determining general tolerances in species are difficult to directly compare between species. This is because, as found in this study, values change depending on developmental stage. However, the critical oxygen tension values of *N. burrowsius*

do appear lower overall than other studied species. For example, Atlantic salmon (*Salmo salar*) eggs had an approximate value of 95 torr at 10 °C, early stage northern pike (*Esox lucius*) eggs had a critical oxygen tension of 30 torr at 20 °C, and at a later stage 50 torr at 14 °C (Blaxter 1969). These comparisons indicate that *N. burrowsius* eggs have a low metabolic rate as they are able to regulate oxygen consumption to very low levels.

There are two common responses of embryos that develop at reduced dissolved oxygen levels; being retarded development and premature hatching (Viljanen & Koho 1991; Keckeis et al. 1996). Both of these modifications in hatching time were observed in *N. burrowsius* eggs. Further, it has been widely shown that temperature and oxygen supply influence the time to hatching in fish (Blaxter 1969). Moreover, hatching can be delayed by extreme reductions in dissolved oxygen, irrespective of the development stage at which embryos are exposed (Keckeis et al. 1996). However, although most significant results are from situations of extreme oxygen stress, mild stressors can still have subtle detrimental effects on development (Wiegand et al. 1989). For example, although adult channel catfish (*Ichtalurus punctatus*) are tolerant of hypoxia, embryos were sensitive to small reductions in dissolved oxygen (70 % saturation; Carlson et al. 1974). Furthermore, stress during reduced oxygen saturation can be worsened by elevated temperature, e.g. although lake trout (*Salvelinus namaycush*) fry survived various reduced oxygen levels at 7 °C they did not at 10 °C (Carlson & Siefert 1974). The present study found that *N. burrowsius* eggs could develop and survive until hatching at a temperature of 20 °C, although only those eggs in well oxygenated positions were fully developed at hatching.

Most embryos raised at 20 °C and at reduced oxygen levels showed signs of abnormal or premature development. The most prevalent malformation involved spinal curvature. Body deformities are a common response of fish embryos to reduced oxygen (Keckeis et al. 1996; Einum et al. 2002). However, abnormal body curvatures have also been induced by low temperatures in goldfish (*Carassius auratus*; Wiegand et al. 1989), and inbreeding in rainbow trout (*Oncorhynchus mykiss*; Aulstad & Kittelsen 1971). Genetic diversity is likely to be an important consideration when evaluating deformity rates as adverse environmental conditions can exacerbate underlying genetic vulnerabilities. This may be the case in *N. burrowsius* as it has low heterozygocity and adult deformity, attributed to both genetic and environmental causes occurs (Davey et al. 2003; Chapter 6). However, deformities of embryos are difficult to assess in the wild as few survive past yolk absorption (Keckeis et al. 1996).

This study however was not able to differentiate between permanent malformation and transient effects of premature hatching. Exposure to hypoxia is a highly effective inducer of premature hatching and can be used to synchronise hatching in aquaculture (Oppen-Berntsen et al. 1990). In later developmental stages embryos can be very active, especially just prior to hatching (Blaxter 1969; Keckeis et al. 1996). Movement may facilitate oxygen distribution within the egg, thus increased movement during oxygen stress could promote chorion rupture, which will also be weakened by the action enzymes (Viljanen & Koho 1991). Premature hatching is likely to be a mechanism of improving oxygen absorption by the embryo. In discarding the chorion the embryo will not be affected by limits imposed by chorion permeability (Viljanen & Koho 1991), which may also decrease as eggs accumulate debris. Furthermore, movement of the embryo, although very limited, is possible without a hindering chorion. This may enable embryos to move into more oxygenated water or to increase water circulation in their vicinity. Thus, premature hatching is likely to increase oxygen absorption, while the embryo continues to develop on yolk reserves, as seen in the embryos of the annual killifish species *Nothobranchius korthausae* which in late development stages regularly escape hypoxia by hatching (Levels et al. 1986). However, hatching before locomotory function is fully developed may increase vulnerability to predation.

In summary, *N. burrowsius* embryos demonstrated physiological and behavioural responses including the possible occurrence of a quiescent state, which are likely to moderate the effects of exposure to hypoxia. However, exposure of eggs to hypoxia and elevated temperatures are still likely to have adverse consequences, including reduced yolk-conversion efficiency leading to smaller fry (Bams & Lam 1983). Further, the survival of prematurely hatched *N. burrowsius* fry is at present uncertain. Thus, factors which delay spawning of *N. burrowsius* and result in the exposure of eggs to hypoxic summer conditions could suppress recruitment.

Chapter 12.

General discussion.

Population regulation, through density-dependent mechanisms, may be essential for long-term persistence of a population (Hixon et al. 2002). Canterbury mudfish (*Neochanna burrowsius*) populations at low densities are capable of rapid growth because of high fecundity (Eldon 1979c), and are regulated at high densities by mechanisms identified in this study, such as cannibalism and juvenile suppression (Chapter 5). Persistence of *N. burrowsius* populations in disturbed habitats also necessitates resistance to progressively adverse conditions, such as agricultural intensification, and resilience after acute disturbance, such as severe drought. Despite dire predictions of their likely demise (Skrzynski 1968); *N. burrowsius* has endured the agricultural transformation of the formerly wetland-dominated Canterbury Plains. This has mainly been due to intrinsic traits, including vagility and physiological tolerance to environmental extremes (Eldon et al. 1978; Eldon 1979d; Meredith 1985). However, some populations are still vulnerable and the intrinsic characteristics of *N. burrowsius* may be insufficient to ensure long term persistence if extrinsic processes, such as intensification of land use, continue (Chapters 4, 5 & 6). Furthermore, recent modelling of climate trends strongly suggests that drought frequency will increase by between two and four-fold by 2080 across the Canterbury Plains (Mullan et al. 2005). This is likely to lead to further extinctions of *N. burrowsius* populations as they not specifically adapted to extended drought conditions (Eldon 1979a; Meredith 1985).

In this thesis I focused on *N. burrowsius* populations within the four most important natural habitats identified by Eldon (1993). Despite the importance of these, two sites had characteristics that suggested that the long-term security of the *N. burrowsius* populations they contain is in doubt. The Hororata Spring population, in particular, seems vulnerable to extirpation (Chapter 2). This habitat experienced the greatest hydrological fluctuations, high agricultural disturbance, and both shortfin eel (*Anguilla australis*) and upland bully (*Gobiomorphus breviceps*) were present. Likely as a consequence, the *N. burrowsius* population had a low density, small individual size, and high rates of pathogenic infection (Chapters 5 & 6). Conversely, comparison with other NZFFD records for *N. burrowsius* sites indicates that my Gee minnow catch per unit effort results at Te Roto Repo o Tawera and St

Andrews Drain were at the upper end of those recorded previously (Figure 12.1a). Furthermore, the maximum length of *N. burrowsius* found at these two sites was greater than that previously recorded in the NZFFD (Figure 12.1b). Maximum *N. burrowsius* length is important as fecundity increases substantially with increasing size (Eldon 1979c).

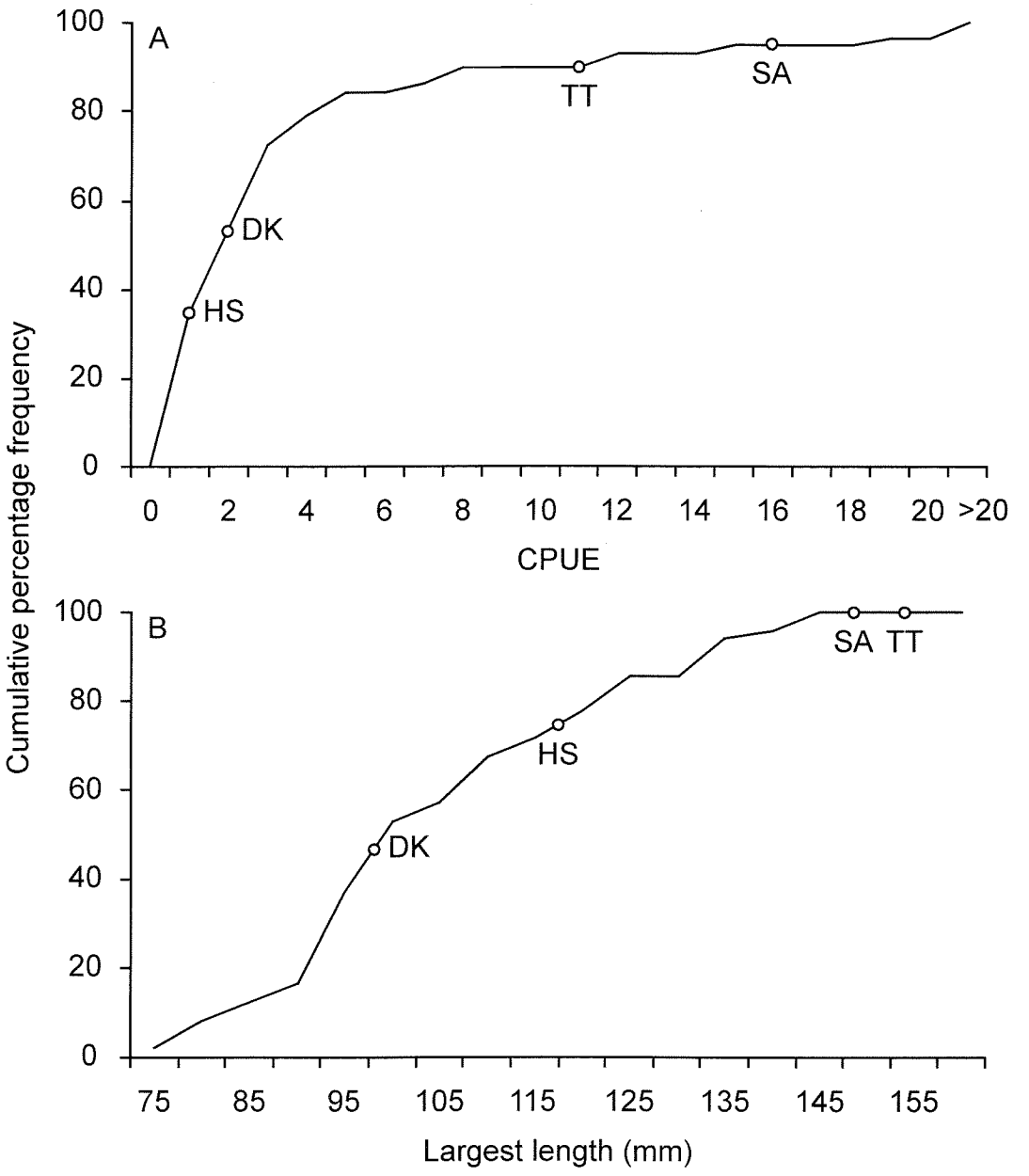

Figure 12.1. Cumulative percentage frequency of (a), 58 *N. burrowsius* CPUE records using Gee minnow traps only, and (b), the length of the largest *N. burrowsius* recorded from 49 records obtained from the NZFFD on 16 May 2005. CPUE results and length of the largest *N. burrowsius* collected during April 2002 for sites studied in this thesis are overlaid: TT = Te Roto Repo o Tawera, HS = Hororata Spring, SA = St Andrews Drain, DK = Dog Kennel Stream. Note the NZFFD records include previous records for the sites studied in this thesis and multiple temporal entries for locations.

At each of my four study sites the fish community and the level of hydrological and agricultural disturbance was different (Table 1.2). In total, hydrological regime and the presence of *A. australis* had a greater effect on population characteristics than the presence of *G. breviceps*, geographical location, and agriculture (Chapter 5). Agriculture is widely recognised as an activity that can limit fish communities, due to channelisation of waterways (Portt et al. 1986), reduced water quality (Hickey et al. 1989; Eklöv et al. 1998), and disturbance from mechanical macrophyte clearance (Hudson & Harding 2004). However, I did not find strong evidence during my study that the level of direct agricultural activity occurring at the four sites studied was a significant factor structuring *N. burrowsius* populations. For instance, although the Dog Kennel Stream site was fenced and re-vegetated, it contained a *N. burrowsius* population that appeared constrained, consisting of small individuals at low density (Chapter 5). In comparison, St Andrews Drain, which was a channelised drainage ditch that received stock grazing and mechanical macrophyte clearance, contained a population consisting of large individuals at high densities. Nonetheless, pathogenic infection was more prevalent in the two sites with higher nitrate concentrations and greater stock access to the waterway (Chapter 6). High stock levels or access to waterways may increase bacteria levels and reduce water and habitat quality, leading to impaired fish health and increased susceptibility to pathogenic infection. As *N. burrowsius* is considered a 'clean water species' (McDowall 1998), it is likely that if water quality continues to decline at these sites this situation will worsen, possibly leading to high mortality rates and population extirpation. Another consideration is that beef cattle only began to be stocked at St Andrews Drain in 2002. It is likely that there is a lag period between habitat degradation due to stock damage and an observable change to fish populations. However, even in the short term, removal of macrophytes by beef cattle altered the within-site distribution of *N. burrowsius* (Chapter 4). Macrophytes provide essential habitat for *N. burrowsius* and the presence of particular plant species was associated with the presence of adults and eggs (Chapters 3 & 10). In some situations, plant removal may not have a detrimental effect on populations, if there is sufficient habitat remaining, or if macrophyte loss is short term because of vigorous re-growth. However, removal of the cover provided by aquatic plants, by grazing and/or mechanical removal may increase predation risk by herons (*Ardea novaehollandiae*) and limit recruitment if clearance occurs immediately prior to or during the spawning period (Chapter 10). Furthermore, the consequences of aquatic plant removal may be exacerbated if *G. breviceps* are present and displace *N. burrowsius* from remaining refuge (Chapter 8).

Consequences of drought and hypoxia

Neochanna species are associated with aquatic habitats that experience drought disturbance (Skrzynski 1968; Eldon 1979a). However, extensive and repeated drying of *N. burrowsius* habitats is likely to be a relatively recent occurrence exacerbated by wetland drainage, agricultural activities, and climatic change (Eldon et al. 1978; McDowall 1998; Mullan et al. 2005). The findings of this thesis support the views of Eldon (1979a) and Meredith (1985) that *N. burrowsius* are not particularly well adapted to surviving periods of complete or extended habitat desiccation. Instead, adaptations likely evolved to enhance survival in periodically hypoxic wetlands, such as air breathing and an amphibious tendency, have fortuitously promoted survival during periods of drought (Eldon 1979a; Meredith 1985). Despite none of my study sites becoming completely desiccated, as small pools of water always remained, high mortality of emersed *N. burrowsius* was recorded (Chapter 7). Further, in my field study, the occurrence of intermittent flow during summer led to delayed *N. burrowsius* spawning the following spring, as indicated by egg stage (Chapter 9). Additionally, during experimental investigations, low dissolved oxygen levels delayed spawning in *N. burrowsius* (Chapter 9). These responses would result in a reduced time available for egg development and fry growth before the onset of summer conditions. This is likely to have important consequences for recruitment in drought disturbed habitats, as individuals less than approximately 50 mm TL suffered high mortality when emersed (Chapter 7). The congeners, brown mudfish (*Neochanna apoda*) and black mudfish (*N. diversus*) that occur in habitats that routinely dry up completely, spawn in autumn (Eldon 1978; Town 1981). This earlier spawning allows a longer period of egg and fry development before subsequent re-drying of habitat. The lack of appropriate spawning timing in *N. burrowsius* is thus likely to increase its susceptibility to increasing drought severity.

Inter-specific interactions

In general, it is considered that *Neochanna* spp. have a realised niche bounded by environmental factors and negative inter-specific interactions (McDowall 1990). This is likely due to the general trade-off between competitive ability and the capacity to survive environmental disturbance (Begon et al. 1990). In some circumstances, periods of hypoxia and drought are considered beneficial to *Neochanna* populations (Hicks & Barrier 1996). This is because deteriorating dissolved oxygen levels deleteriously affects co-occurring fish species to a greater extent than the physiologically more tolerant *Neochanna* (Eldon 1979a; McDowall 1990). Occurrence of *Neochanna* species in seasonally drying wetlands is

common (Eldon 1978; McDowall 1990; Hicks & Barrier 1996). A survey found that 87 % of *N. diversus* sites in the Waikato experienced desiccation, affording *N. diversus* protection from introduced Gambusia (*Gambusia affinis*; Hicks & Barrier 1996). In my study, *N. burrowsius* was only found with predatory *A. australis* in sites with hydrological fluctuations and not in the more benign perennially flowing sites. Other *N. burrowsius* sites containing *A. australis* also periodically dry up (Glova & Hulley 1998). It is likely that without hydrological disturbance, *A. australis* may be able to extirpate *N. burrowsius*. Indeed, I found that the presence of *A. australis* in a habitat reduced the abundance of *N. burrowsius* (Chapters 2 & 4). Further, *A. australis* may suffer higher levels of mortality than *N. burrowsius* during drought and may not be as resilient after disturbance (Stokell 1949; Glova & Hulley 1998). Thus, it is possible that disturbance mediates co-existence of these species, in a way similar to that described by Closs & Lake (1996) for mountain galaxias (*Galaxias olidus*) and brown trout (*Salmo trutta*). However, habitat drying and hypoxia will only have ecological benefits if potential competitors or predators are present or likely to invade during more benign conditions. Otherwise drought disturbance is simply a limiting factor, as high mortality can be incurred, and it can also have deleterious effects on growth and reproduction.

Contrary to expectations, competition between *N. burrowsius* and *G. breviceps* was not as asymmetrical as suggested in the literature (Eldon 1979a; Meredith 1985; McDowall 1990). Various mechanisms promoted co-existence between *N. burrowsius* and *G. breviceps*, including temporal segregation, differing diet composition, and physiological tolerance to environmental extremes by *N. burrowsius*. However, other effects found in my study, such as displacement from refuge, increased activity, and altered energy allocation, are still likely to have consequences for *N. burrowsius* survival, fecundity and thus fitness. Furthermore, habitats dominated by silt, and not cobbles may favour *N. burrowsius* and negatively influence *G. breviceps* populations. The ratio between silt and cobble substratum is largely controlled by hydrological factors, which as mentioned, have been altered on the Canterbury Plains. Although relatively depauparate, the composition of the fish community at the sites studied changed over the course of the study, and differed from previous studies, with both local extinction and species invasion occurring. At Dog Kennel Stream in 1984 three *G. breviceps* were recorded as being caught with a hand net, along with *N. burrowsius* (NZFFD record number 6639). Eldon (1993, p. 7) described Dog Kennel Stream as containing *G. breviceps*, but that they were rare, and the waterway to be 'free of immigration by diadromous fish' due to downstream channelisation and dry sections inhibiting dispersal. In contrast, I did not find *G. breviceps* but captured *A. australis* at this site. Furthermore, at St Andrews Drain

in 1976, *G. breviceps* and common bully (*Gobiomorphus cotidianus*) were recorded as being present, with *G. breviceps* being noted as common (NZFFD card numbers 50714 and 50715). In 1983 *G. breviceps* was still present (NZFFD card number 5662). In 1999, nine *G. breviceps* were caught, all being very large adults. But in 2002 only one large *G. breviceps* was caught, and it was considered that this population had effectively become extinct. It is likely that this was due to the lack of suitable cobble spawning habitat for *G. breviceps* at this site. These changes in fish community are likely to be a reflection of the on-going changes in the hydrology and land-use of the Canterbury Plains. The long-term effect of these changes is unknown, but they could potentially have major affects on *N. burrowsius* populations. For example, Eldon (1979a) detailed changes to the hydrology and morphology of a habitat that previously contained *N. burrowsius*, when reported on by Stokell (1949), but that contained only *S. trutta* when revisited.

Physiological and behavioural plasticity

In my studies *N. burrowsius* were remarkably adaptable and responsive to their environment. Both field and experimental studies found that *N. burrowsius* altered how energy resources were allocated, leading to reduced growth in length, yet maintenance or increase in body mass (Chapter 5 & 8). This occurred in response to the presence of a competitor and at sites that regularly dried up or became hypoxic, necessitating periods of emersion. In both situations it is likely that retaining condition instead of growing in length, which is largely irreversible, will allow resources to be diverted to fulfil other requirements, such as sustaining periods of starvation, avoiding inter-specific interactions and/or increased allocation to reproduction. Further, female *N. burrowsius* exposed to differing environmental conditions immediately prior to spawning were able to manipulate the trade-off between the number and size of eggs (Chapter 9). Embryos could also regulate oxygen consumption and survive short periods of extreme hypoxia (Chapter 11). Such compensatory mechanisms can mediate environmental influences, and can determine a population's persistence in the face of disturbance (Le Cren 1965). However, reduced size is likely to have consequences for fecundity potential and fitness. Thus, in drought-disturbed habitats, mortality, suspension of growth and physiological constraints on adult size, may limit population increases via decreased total population fecundity, even though reproductive allocation remains high. Moreover, small size will mean that *N. burrowsius* remain vulnerable to predation by *A. australis*.

Will *N. burrowsius* persist on the Canterbury Plains?

There are many aspects that generate concern for the long-term likelihood of *N. burrowsius* persistence, such as stunting, high rates of pathogenic infection, deformity, fluctuating abundances, and regional synchronicity. It is likely that *N. burrowsius* populations will be increasingly adversely affected if the frequency of droughts increase, as has been predicted (Mullan et al. 2005), or if dissolved nitrate concentrations and stock levels increase. Overall, *N. burrowsius* will only persist in the wild in perpetuity with a concerted effort and changes in behaviour on the part of humans. Since *N. burrowsius* occurs predominately within an agriculture-dominated landscape, human actions directly influence its survival (Eldon 1979d). Early settlers spent considerable effort and capital removing *N. burrowsius* habitat, commenting that the land was 'a sink for money' (McDowall 1998, p. 35). Accounts describe 'gangs of men ... digging their way through the swampy peats and clays "to free the squelching soil from water"' (McDowall 1998, p. 36). It took about 40 years of effort, but they succeeded. Today, little evidence of the vast wetlands of the Canterbury Plains remains. A similar investment, in fact most likely less, is now needed to ensure that the unique fauna that once occurred in these wetlands is retained. This study showed that even in their most important habitats, populations of *N. burrowsius* exhibited characteristics consistent with their acutely threatened status. Furthermore, simply due to the high level of population fragmentation they have experienced and their low genetic diversity, *N. burrowsius* is vulnerable to extinction. We cannot afford to lose any more *N. burrowsius* populations through the purposeful removal of habitat, for example by stockwater reticulation (Chapter 1). Nor can we afford to be inactive as water quantity and quality on the Canterbury Plains gradually declines. There is an increasing awareness that *N. burrowsius* has endured the complete transformation of its habitat and that it can persist in an agricultural setting. Thus, it may take very little to protect the species, requiring in many cases only a willingness to spend the time to understand its requirements. However, in what ever form, an investment is needed now to protect the last fragments of habitat suitable for *N. burrowsius*. Why? Possibly because species extinction would affect New Zealand's image as a 'clean green' nation, the cost of which is valued at more than $500 million (MfE 2001). Or more simply, because as aptly put by Tony Eldon 'life will be boring when all the small creatures have gone' (Eldon 1979d, p. 19).

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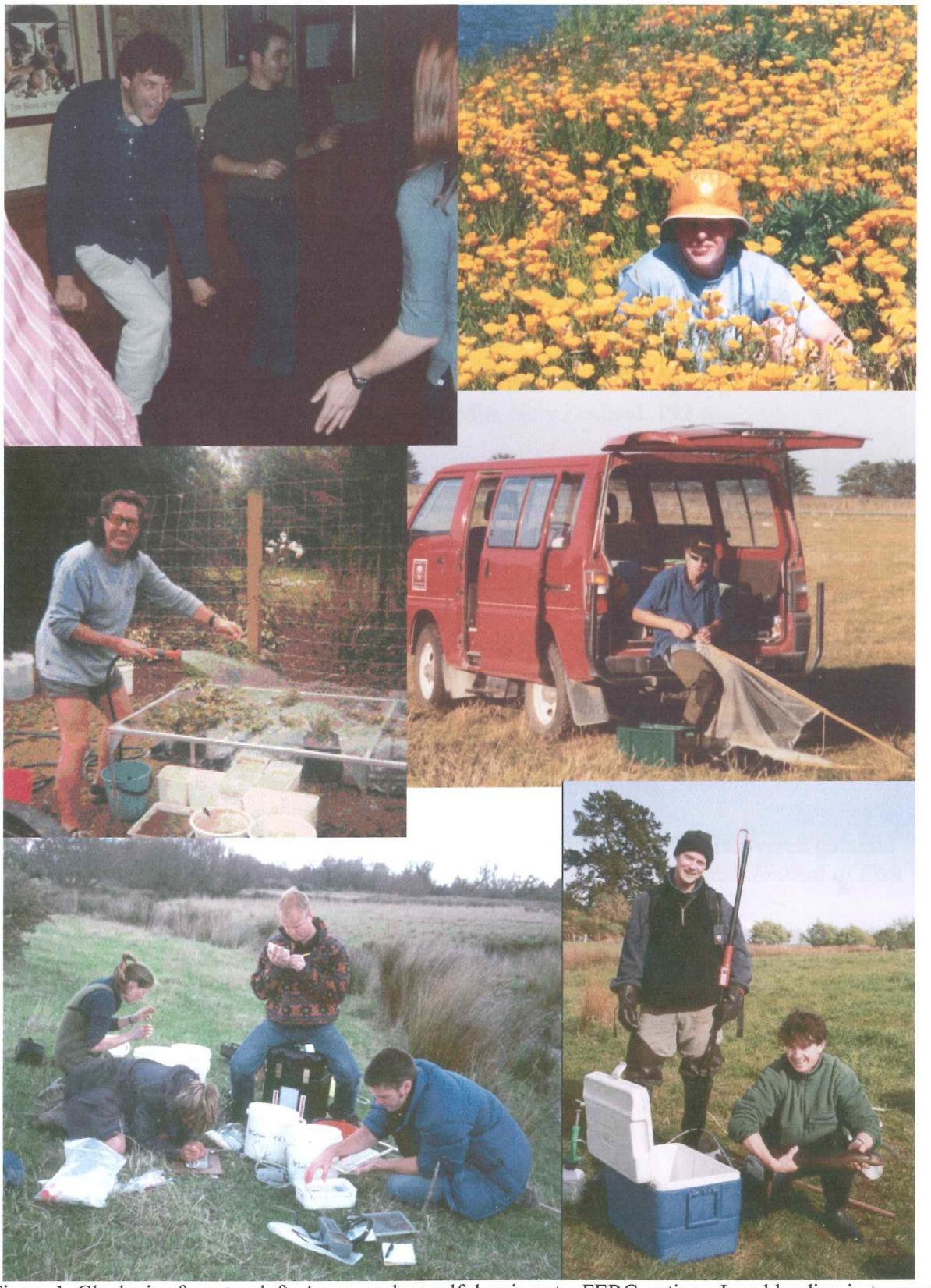

Figure 1. Clockwise from top left: Angus and myself dancing at a FERG outing. Jane blending in to sneak up on mudfish fry. Nicholas fixing a net in the FERG van. Aslan and Chris with the catch of the day. Myself, Marlene, Hans, and Greg processing fish. Russell cleaning macrophytes before an experiment.

REFERENCES

Adams, C.E.; Huntingford, F.A. 2004: Incipient speciation driven by phenotypic plasticity? Evidence from sympatric populations of Arctic charr. *Biological Journal of the Linnean Society 81*: 611-618.

Aitchison-Earl, P.; Chater, M.; Dicker, M.; Ettema, M.; Sanders, R.; Scott, D.; Weeber, J. 2001: State of the Canterbury Region water resource, 1 October 2001. Environment Canterbury, Christchurch, New Zealand. 58 p.

Albanese, B.; Angermeier, P.L.; Dorai-Raj, S. 2004: Ecological correlates of fish movement in a network of Virginia streams. *Canadian Journal of Fisheries and Aquatic Sciences 61*: 857-869.

Allibone, R.M. 1997: Ecology and distribution of the Taieri River galaxiids. Unpublished PhD thesis, University of Otago, Dunedin, New Zealand. 192 p.

Allibone, R.M. 2000: Water abstraction impacts on the non-migratory galaxiids of Totara Creek. *Science for Conservation 147B*: 25-45.

Allibone, R.M. 2003: Egg cannibalism by inanga (*Galaxias maculatus*). *New Zealand Journal of Marine and Freshwater Research 37*: 763-765.

Allibone, R.M.; Caskey, D. 2000: Timing and habitat of koaro (*Galaxias brevipinnis*) spawning in streams draining Mt Taranaki, New Zealand. *New Zealand Journal of Marine and Freshwater Research 34*: 593-595.

Allibone, R.M.; Chadderton, W.L. 1992: Marmite, a new alternative fish bait. *Freshwater Catch 48*: 19.

Allibone, R.M.; Townsend, C.R. 1997a: Distribution of four recently discovered galaxiid species in the Taieri River, New Zealand: the role of microhabitat. *Journal of Fish Biology 51*: 1235-1246.

Allibone, R.M.; Townsend, C.R. 1997b: Reproductive biology, species status and taxonomic relationships of four recently discovered galaxiid fishes in a New Zealand river. *Journal of Fish Biology 51*: 1247-1261.

Allibone, R.M.; Townsend, C.R. 1998: Comparative dietary analysis of a recently described fish complex (Galaxiidae) in a New Zealand river. *New Zealand Journal of Marine and Freshwater Research 32*: 351-361.

Alvarez-León, R. 1980: A specimen of *Lutjanus argentiventris* (Peters) lacking pelvic fins. *Journal of Fish Biology 16*: 563-564.

Anderson, C.S. 1995: Measuring and correcting for size selection in electrofishing and mark-recapture experiments. *Transactions of the American Fisheries Society 124*: 663-676.

Anderson, R.O.;Gutreuter, S.J. 1983: Length, weight, and associated structural indices. Pp. 283-300 *in:* L.A. Nielsen; D.L. Johnson (editors) *Fisheries techniques*, American Fisheries Society, Maryland, United States of America.

Andrews, A.P. 1991: Observations on the Tasmanian mudfish, *Galaxias cleaveri* (Pisces: Galaxiidae). *Papers and Proceedings of the Royal Society of Tasmania 125*: 55-59.

Angermeier, P.L. 1995: Ecological attributes of extinction-prone species: loss of freshwater fishes of Virginia. *Conservation Biology 9*: 143-158.

Atkinson, R.J.A.; Pelster, B.; Bridges, C.R.; Taylor, A.C.; Morris, S. 1987: Behavioural and physiological adaptations to a burrowing life style in the snake blenny, *Lumpenus lampretaeformis,* and the red band-fish, *Cepola rubescens*. *Journal of Fish Biology 31*: 639-659.

Aulstad, D.; Kittelsen, A. 1971: Abnormal body curvatures of rainbow trout (*Salmo gairdneri*) inbred fry. *Journal of the Fisheries Research Board of Canada 28*: 1918-1920.

Bagenal, T.B. 1969a: The relationship between food supply and fecundity in brown trout *Salmo trutta* L. *Journal of Fish Biology 1*: 167-182.

Bagenal, T.B. 1969b: Relationship between egg size and fry survival in brown trout *Salmo trutta* L. *Journal of Fish Biology 1*: 349-353.

Bagenal, T.B. 1972: The variability in numbers of perch, *Perca fluviatilis* L. caught in traps. *Freshwater Biology 2*: 27-36.

Baguette, M.; Schtickzelle, N. 2003: Local population dynamics are important to the conservation of metapopulations in highly fragmented landscapes. *Journal of Applied Ecology 40*: 404-412.

Baker, C.F.; Jowett, I.G.; Allibone, R.M. 2003: Habitat use by non-migratory Otago galaxiids and implications for water management. *Science for Conservation 221*. 34 p.

Bams, R.A.; Lam, C.N.H. 1983: Influence of deteriorating water quality on growth and development of chum salmon (*Oncorhynchus keta*) larvae in a Japanese-style keeper channel. *Canadian Journal of Fisheries and Aquatic Sciences 40*: 2098-2104.

Barber, I.; Hoare, D.J.; Krause, J. 2000: Effects of parasites on fish behaviour: a review and evolutionary perspective. *Reviews in Fish Biology and Fisheries 10*: 131-165.

Barber, I.; Ruxton, G.D. 1998: Temporal prey distribution affects the competitive ability of parasitized sticklebacks. *Animal Behaviour 56*: 1477-1483.

Barnett, B.S.; Schneider, R.S. 1974: Fish populations in dense, submersed plant communities. *Hyacinth Control Journal 12*: 12-14.

Barrier, R.F.G.; Hicks, B.J. 1994: Behavioural interactions between black mudfish (*Neochanna diversus* Stokell, 1949: Galaxiidae) and mosquitofish *Gambusia affinis* (Baird & Girard, 1854). *Ecology of Freshwater Fish 3*: 93-99.

Barrier, R.F.G.; Hicks, B.J.; Dean, T.L.; Ling, N. 1996: Habitat requirements and aerial respiration in the black mudfish. *Water & Atmosphere 4(3)*: 20-22.

Begon, M.; Harper, J.L.; Townsend, C.R. 1990: *Ecology: individuals, populations and communities. Second edition.* Blackwell Scientific Publications, Cambridge, Massachusetts, United States of America. 945 p.

Belovsky, G.E.; Mellison, C.; Larson, C.; van Zandt, P.A. 1999: Experimental studies of extinction dynamics. *Science 286*: 1175-1177.

Bennett, P.M.; Owens, I.P.F. 1997: Variation in extinction risk among birds: chance or evolutionary predisposition? *Proceedings of the Royal Society of London B 264*: 401-408.

Benton, T.G. 2003: Understanding the ecology of extinction: are we close to the critical threshold? *Annales Zoologici Fennici 40*: 71-80.

Benzie, V.L. 1961: A comparison of the life history and variation in two species of *Galaxias*, *G. attenuatus* and *G. vulgaris*. Unpublished MSc thesis, University of Canterbury, Christchurch, New Zealand. 233 p.

Benzie, V.L. 1968: Stages in the normal development of *Galaxias maculatus attenuatus* (Jenyns). *New Zealand Journal of Marine and Freshwater Research 2*: 606-627.

Beraldo, P.; Pinosa, M.; Tibaldi, E.; Canavese, B. 2003: Abnormalities of the operculum in gilthead sea bream (*Sparus aurata*): morphological description. *Aquaculture 220*: 89-99.

Bertschy, K.A.; Fox, M.G. 1999: The influence of age-specific survivorship on pumpkinseed sunfish life histories. *Ecology 80*: 2299-2313.

Blair, D.; 1984. 1984: A checklist and bibliography of parasites of New Zealand freshwater fish. *Mauri Ora 11*: 5-50.

Blaxter, J.H.S. 1969: Development: eggs and larvae. Pp. 177-252 *in:* W.S. Hoar; D.J. Randall (editors.) *Fish physiology. Volume III. Reproduction and growth, bioluminescence, pigments, and poisons*, Academic Press, New York, United States of America.

Bodensteiner, L.R.; Sheehan, R.J.; Willis, P.S.; Brandenburg, A.M.; Lewis, W.M. 2000: Flowing water: an effective treatment for ichthyophthiriasis. *Journal of Aquatic Animal Health 12*: 209-219.

Bohlen, J. 2003: Temperature and oxygen requirements of early life stages of the endangered spined loach, *Cobitis taenia* L. (Teleostei, Cobitidae) with implications for the management of natural populations. *Archiv-für-Hydrobiologie 157*: 195-212.

Bolger, T.; Connolly, P.L. 1989: The selection of suitable indices for the measurement and analysis of fish condition. *Journal of Fish Biology 34*: 171-182.

Bond, N.R.; Lake, P.S. 2003: Characterizing fish–habitat associations in streams as the first step in ecological restoration. *Austral Ecology 28*: 611-621.

Bonner, T.H.; Brandt, T.M.; Fries, J.N.; Whiteside, B.G. 1998: Effects of temperature on egg production and early life stages of the fountain darter. *Transactions of the American Fisheries Society 127*: 1998.

Bonnett, M.L. 1992: Spawning in sympatric alpine galaxias (*Galaxias paucispondylus* Stokell) and longjawed galaxias (*G. prognathus* Stokell) in a South Island, New Zealand, high-country stream. *New Zealand Natural Sciences 19*: 27-30.

Bonnett, M.L.; Lambert, P.W. 2002: Diet of giant kokopu, *Galaxias argenteus*. *New Zealand Journal of Marine and Freshwater Research 36*: 361-369.

Bourne, J. 2000: Louisiana's vanishing wetlands: going, going ... *Science 289*: 1860-1863.

Boyce, M.S. 1992: Population viability analysis. *Annual Review in Ecology Systematics 23*: 481-506.

Broad, T.L.; Townsend, C.R.; Closs, G.P.; Jellyman, D.J. 2001: Microhabitat use by longfin eels in New Zealand streams with contrasting riparian vegetation. *Journal of Fish Biology 59*: 1385-1400.

Brönmark, C.; Miner, J.G. 1992: Predator-induced phenotypical change in body morphology in crucian carp. *Science 258*: 1348-1350.

Brönmark, C.; Paszkowski, C.A.; Tonn, W.M.; Hargeby, A. 1995: Predation as a determinant of size structure in populations of crucian carp (*Carassius carassius*) and tench (*Tinca tinca*). *Ecology of Freshwater Fish 4*: 85-92.

Brown, A.F.; Diamond, M. 1984: The consumption of rainbow trout (*Salmo gairdneri* Richardson) eggs by macroinvertebrates in the field. *Freshwater Biology 14*: 211-215.

Brown, A.F.; Laland, K.N. 2001: Social learning and life skills training for hatchery reared fish. *Journal of Fish Biology 59*: 471-493.

Brown, C.; Laland, K.N. 2002: Social learning of a novel avoidance task in the guppy: conformity and social release. *Animal Behaviour 64*: 41-47.

Brown, E.A.R.; Scott, D.B.C. 1987: Abnormal pelvic fins in Scottish powan, *Coregonus lavaretus* (L.) (Salmonidae, Coregoninae). *Journal of Fish Biology 31*: 443-444.

Brungs, W.A. 1971: Chronic effects of low dissolved oxygen concentrations on the fathead minnow (*Pimephales promelas*). *Journal of the Fisheries Research Board of Canada 28*: 1119-1123.

Burks, R.L.; Lodge, D.M.; Jeppesen, E.; Lauridsen, T.L. 2002: Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. *Freshwater Biology 47*: 343-365.

Burleson, M.L.; Wilhelm, D.R.; Smatresk, N.J. 2001: The influence of fish size on the avoidance of hypoxia and oxygen selection by largemouth bass. *Journal of Fish Biology 59*: 1336-1349.

Burnet, A.M.R. 1965: Observations on the spawning migrations of *Galaxias attenuatus* (Jenyns). *New Zealand Journal of Science 8*: 79-87.

Buxton, R. 1991: New Zealand's wetlands: a management guide, Department of Conservation, Wellington, New Zealand. 102 p.

Bye, V.J. 1984: The role of environmental factors in the timing of reproductive cycles. Pp. 187-205 *in:* G.W. Potts; R.J. Wootton (editors) *Fish reproduction: strategies and tactics*, Academic Press, London, United Kingdom.

Cadwallader, P.L. 1973: The ecology of *Galaxias vulgaris* (Pisces: Salmoniformes: Galaxiidae) in the River Glentui, Canterbury, New Zealand. Unpublished PhD thesis, University of Canterbury, Christchurch, New Zealand. 211 p.

Cadwallader, P.L. 1975a: Distribution and ecology of the Canterbury mudfish, *Neochanna burrowsius* (Phillipps) (Salmoniformes: Galaxiidae). *Journal of the Royal Society of New Zealand 5*: 21-30.

Cadwallader, P.L. 1975b: A laboratory study of interactive segregation between two New Zealand stream-dwelling fish. *Journal of Animal Ecology 44*: 865-875.

Cadwallader, P.L. 1975c: Feeding habits of two fish species in relation to invertebrate drift in a New Zealand river. *New Zealand Journal of Marine and Freshwater Research 9*: 11-26.

Cadwallader, P.L. 1976: Breeding biology of a non-diadromous galaxiid, *Galaxias vulgaris* Stokell, in a New Zealand river. *Journal of Fish Biology 8*: 157-177.

Campana, S.E.; Casselman, J.M. 1993: Stock discrimination using otolith shape analysis. *Canadian Journal of Fisheries and Aquatic Sciences 50*: 1062-1083.

Cardillo, M. 2003: Biological determinants of extinction risk: why are smaller species less vulnerable? *Animal Conservation 6*: 63-69.

Cargnelli, L.M.; Gross, M.R. 1997: Fish energetics: larger individuals emerge from winter in better condition. *Transactions of the American Fisheries Society 126*: 153-156.

Carle, L.C.; Strub, M.R. 1978: A new method for estimating population size from removal data. *Biometrics 34*: 621-630.

Carlson, A.R.; Siefert, R.E. 1974: Effects of reduced oxygen on the embryos and larvae of lake trout (*Salvelinus namaycush*) and largemouth bass (*Micropterus salmoides*). *Journal of the Fisheries Research Board of Canada 31*: 1393-1396.

Carlson, A.R.; Siefert, R.E.; Herman, L.J. 1974: Effects of lowered dissolved oxygen concentrations on channel catfish (*Ictalurus punctatus*) embryos and larvae. *Transactions of the American Fisheries Society 3*: 623-626.

Casselman, J.M.; Harvey, H.H. 1975: Selective fish mortality resulting from low winter oxygen. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 19*: 2418-2429.

Chapman, A.; Lewis, M. 1976: *An introduction to the freshwater Crustacea of New Zealand.* Collins, Auckland. 261 p.

Chapman, L.J.; Chapman, C.A.; Chandler, M. 1996: Wetland ecotones as refugia for endangered fishes. *Biological Conservation 78*: 263-270.

Chapman, L.J.; Chapman, C.A.; Nordlie, F.G.; Rosenberger, A.E. 2002a: Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. *Comparative Biochemistry and Physiology Part A 133*: 421-437.

Chapman, L.J.; Nordlie, F.G.; Seifert, A. 2002b: Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development. *Journal of Fish Biology 61*: 242-251.

Charteris, S.C.; Allibone, R.M.; Death, R.G. 2003: Spawning site selection, egg development, and larval drift of *Galaxias postvectis* and *G. fasciatus* in a New Zealand stream. *New Zealand Journal of Marine and Freshwater Research 37*: 493-505.

Ciepielewski, W. 1967: Wzrost i Przezywalnosc izolowaneji populacji karasia *Carassius carassius* (L.) – w malym. Nieeksploatowanym jeziore. *Rochz-i Nauk Roln 90*: 239-248. (Original not seen).

Claessen, D.; Van Oss, C.; De Roos, A.M.; Persson, L. 2002: The impact of size-dependent predation on population dynamics and individual life history. *Ecology 83*: 1660-1675.

Clarkson, B.R.; Sorrell, B.K.; Reeves, P.N.; Champion, P.D.; Partridge, T.R.; Clarkson, B.D. 2004: Handbook for monitoring wetland condition - coordinated monitoring of New Zealand wetlands. Ministry for the Environment, Wellington, New Zealand. 73 p.

Closs, G.P.; Lake, P.S. 1996: Drought, differential mortality and the coexistence of a native and an introduced fish species in a south east Australian intermittent stream. *Environmental Biology of Fishes 47*: 17-26.

Cloud, J.G.; Erdahl, A.L.; Graham, E.F. 1988: Survival and continued normal development of fish embryos after incubation at reduced temperatures. *Transactions of the American Fisheries Society 117*: 503-506.

Coffey, B.T.; Clayton, J.S. 1988: *New Zealand waterplants: a guide to plants found in New Zealand freshwaters*. Ruakura Agricultural Centre, Ministry of Agriculture and Fisheries, Hamilton, New Zealand. 63 p.

Collins, N.C.; Harvey, H.H.; Tierney, A.J.; Dunham, D.W. 1983: Influence of predatory fish density on trapability of crayfish in Ontario lakes. *Canadian Journal of Fisheries and Aquatic Sciences 40*: 1820-1828.

Cone, R.S. 1989: The need to reconsider the use of condition indices in fishery science. *Transactions of the American Fisheries Society 118*: 510-514.

Cooper, A.B.; Cooke, J.G. 1984: Nitrate loss and transformation in 2 vegetated headwater streams. *New Zealand Journal of Marine and Freshwater Research 18*: 441-450.

Coward, K.; Bromage, N.R.; Little, D.C. 1998: Inhibition of spawning and associated suppression of sex steroid levels during confinement in the substrate-spawning *Tilapia zillii*. *Journal of Fish Biology 52*: 152-165.

Cromarty, P.; Scott, D.A. 1996: A directory of wetlands in New Zealand. Department of Conservation, Wellington, New Zealand. 395 p.

Crook, D.A. 2004: Movements associated with home-range establishment by two species of lowland river fish. *Canadian Journal of Fisheries and Aquatic Sciences 61*: 2183-2193.

Crooks, K.R.; Suarez, A.V.; Bolger, D.T.; Soulé, M.E. 2001: Extinction and colonization of birds on habitat islands. *Conservation Biology 15*: 159-172.

Cross, D.G.; Stott, B. 1975: The effect of electric fishing on the subsequent capture of fish. *Journal of Fish Biology 7*: 349-357.

Dahlberg, M.D. 1970: Frequencies of abnormalities in Georgia estuarine fishes. *Transactions of the American Fisheries Society 99*: 95-97.

Davenport, J.; Lönning, S. 1980: Oxygen uptake in developing eggs and larvae of the cod, *Gadus morhua* L. *Journal of Fish Biology 16*: 249-256.

Davey, M.L.; O'Brien, L.K.; Ling, N.; Gleeson, D.M. 2003: Population genetic structure of the Canterbury mudfish (*Neochanna burrowsius*): biogeography and conservation implications. *New Zealand Journal of Marine and Freshwater Research 37*: 13-21.

Davidson, C.M. 1999: Morphological specialisations for air breathing and aestivation physiology in the black mudfish (*Neochanna diversus* Stokell, 1949). Unpublished MSc thesis, University of Waikato, Hamilton, New Zealand. 102 p.

Davidson, M.M. 1949: Anatomy of *Neochanna apoda* Günther, Galaxiidae. Unpublished MSc thesis, Victoria University College, Wellington, New Zealand. 129 p.

Davis, S. 1984: Regional modifications to waterways. Part III - North Canterbury's aquatic resources. *Freshwater Catch 23*: 10-15.

Dawson, F.H.; Szoszkiewicz, K. 1999: Relationships of some ecological factors with the associations of vegetation in British rivers. *Hydrobiologia 415*: 117-122.

De Szalay, F.A.; Resh, V.H. 2000: Factors influencing macroinvertebrate colonization of seasonal wetlands: responses to emergent plant cover. *Freshwater Biology 45*: 295-308.

Dean, T.L. 1995: Environmental physiology of aquatic and aerial respiration in the black mudfish (*Neochanna diversus* Stokell, 1949). Unpublished MSc thesis, University of Waikato, Hamilton, New Zealand. 120 p.

den Boer, P.J. 1981: On the survival of populations in a heterogeneous and variable environment. *Oecologia 50*: 39-53.

Diamond, M.; Wakefield, P.M. 1986: The consumption of eggs of the perch, *Perca fluviatilis* L., by macroinvertebrates in the field. *Freshwater Biology 16*: 373-376.

Dionne, M.; Folt, C.L. 1991: An experimental analysis of macrophyte growth forms as fish foraging habitat. *Canadian Journal of Fisheries and Aquatic Sciences 48*: 123-131.

DoC (Department of Conservation) 2003: New Zealand mudfish (*Neochanna* spp.) recovery plan 2003 – 13. *Threatened Species Recovery Plan 51*. 25 p.

Domenici, P.; Ferrari, R.S.; Steffensen, J.F.; Batty, R.S. 2002: The effect of progressive hypoxia on school structure and dynamics in Atlantic herring *Clupea harengus*. *Proceedings of the Royal Society of London Part B 269*: 2103-2111.

Downing, J.A. 1991: The effect of habitat structure on the spatial distribution of freshwater invertebrate populations. Pp. 87-106 *in:* S.S. Bell; E.D. McCoy; H.R. Mushinsky (editors) *Habitat structure: the physical arrangement of objects in space*, Chapman & Hall, London.

Dudgeon, D. 2003: Clinging to the wreckage: unexpected persistence of freshwater biodiversity in a degraded tropical landscape. *Aquatic Conservation: Marine and Freshwater Ecosystems 13*: 93-97.

Dudley, T.L. 1988: The roles of plant complexity and epiphyton in colonization of macrophytes by stream insects. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 23*: 1153-1158.

Duffy, C.A.J. 1996: Pre-spawning mortality of koaro (*Galaxias brevipinnis*) in Apias Creek, north-east Ruahine Range, North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research 30*: 403-405.

Dulvy, N.K.; Ellis, J.R.; Goodwin, N.B.; Grant, A.; Reynolds, J.D.; Jennings, S. 2004: Methods of assessing extinction risk in marine fishes. *Fish and Fisheries 5*: 255-276.

Dulvy, N.K.; Reynolds, J.D. 2002: Predicting extinction vulnerability in skates. *Conservation Biology 16*: 440-450.

Duncan, J.R.; Lockwood, J.L. 2001: Extinction in a field of bullets: a search for causes in the decline of the world's freshwater fishes. *Biological Conservation 102*: 97-105.

Dungan, J.L.; Perry, J.N.; Dale, M.R.T.; Legendre, P.; Citron-Pousty, S.; Fortin, M.-J.; Jakomulska, A.; Miriti, M.; Rosenberg, M.S. 2002: A balanced view of scale in spatial statistical analysis. *Ecography 25*: 626-640.

Dunn, N.R. 2003: The effects of extremes in flow on alpine (*Galaxias paucispondylus*) and Canterbury (*G. vulgaris*) galaxias. Unpublished MSc thesis, University of Canterbury, Christchurch, New Zealand. 174 p.

Einum, S.; Hendry, A.P.; Fleming, I.A. 2002: Egg-size evolution in aquatic environments: does oxygen availability constrain size? *Proceedings of the Royal Society of London B 269*: 2325-2330.

Eklöv, A.G.; Greenberg, L.A.; Brönmark, C.; Larsson, P.; Berglund, O. 1998: Response of stream fish to improved water quality: a comparison between the 1960s and 1990s. *Freshwater Biology 40*: 771-782.

Eldon, G.A. 1968: Notes on the presence of the brown mudfish (*Neochanna apoda,* Günther) on the West Coast of the South Island of New Zealand. *New Zealand Journal of Marine and Freshwater Research 2*: 37-48.

Eldon, G.A. 1971: Suspected terrestrial deposition of eggs by *Neochanna apoda* Günther (Pisces: Salmoniformes: Galaxiidae). *Journal of Fish Biology 3*: 247-249.

Eldon, G.A. 1978: The life history of *Neochanna apoda* Günther (Pisces: Galaxiidae). Fisheries Research Bulletin No. 19, New Zealand Ministry of Agriculture and Fisheries, Wellington. 44 p.

Eldon, G.A. 1979a: Habitat and interspecific relationships of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research 13*: 111-119.

Eldon, G.A. 1979b: Food of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research 13*: 255-261.

Eldon, G.A. 1979c: Breeding, growth and aestivation of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research 13*: 331-346.

Eldon, G.A. 1979d: Can the Canterbury mudfish survive? *Catch 6(2)*: 19.

Eldon, G.A. 1983: Mudfish liberations. *Freshwater Catch 18*: 15-16.

Eldon, G.A. 1985: Mudfish, glorious mudfish. *Freshwater Catch 28*: 20.

Eldon, G.A. 1986: Canterbury mudfish in Lake Eldon. *Freshwater Catch 30*: 19.

Eldon, G.A. 1988a: Canterbury mudfish reserves and liberations. Progress report 1987/88. Department of Conservation, Wellington. 3 p.

Eldon, G.A. 1988b: Canterbury mudfish established in new pond. *Freshwater Catch 36*: 7.

Eldon, G.A. 1989: Canterbury mudfish reserves and liberations. Final report 1988/89. Department of Conservation, Wellington. 3 p.

Eldon, G.A. 1992: The difficulties of capturing mudfish. *Freshwater Catch 48*: 16-17.

Eldon, G.A. 1993: Guidelines towards a conservation strategy for the Canterbury mudfish (*Neochanna burrowsius*). New Zealand Freshwater Research Report No. 4. National Institute of Water and Atmospheric Research Ltd, Christchurch. 19 p.

Eldon, G.A.; Field-Dodgson, M.S. 1983: Ponds made from pits. *Freshwater Catch 21*: 7-8.

Eldon, G.A.; Howden, P.J.; Howden, D.B. 1978: Reduction of a population of Canterbury mudfish, *Neochanna burrowsius* (Galaxiidae), by drought. *New Zealand Journal of Marine and Freshwater Research 12*: 313-321.

Engelhardt, K.A.M.; Ritchie, M.E. 2001: Effects of macrophyte species richness on wetland ecosystem functioning and services. *Nature 411*: 687-689.

Essington, T.E.; Kitchell, J.F. 1999: New perspectives in the analysis of fish distributions: a case study on the spatial distribution of largemouth bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Sciences 56 (Supplement 1)*: 52-60.

Fagan, W.F.; Meir, E.; Prendergast, J.; Folarin, A.; Karieva, P. 2001: Characterising population vulnerability for 758 species. *Ecology Letters 4*: 132-138.

Fagan, W.F.; Unmack, P.J.; Burgess, C.; Minkley, W.L. 2002: Rarity, fragmentation, and extinction risk in desert fishes. *Ecology 83*: 3250-3256.

Fahrig, L. 2001: How much habitat is enough? *Biological Conservation 100*: 65-74.

Fausch, K.D.; White, R.J. 1981: Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. *Canadian Journal of Fisheries and Aquatic Sciences 38*: 1220-1227.

Fieberg, J.; Ellner, S.P. 2000: When is it meaningful to estimate an extinction probability? *Ecology 81*: 2040-2047.

Finstad, A.G.; Berg, O.K.; Langeland, A.; Lohrmann, A. 2002: Reproductive investment and energy allocation in an alpine arctic charr, *Salvelinus alpinus*, population. *Environmental Biology of Fishes 65*: 63-70.

Firehock, K.; West, J. 1995: A brief history of volunteer biological water monitoring using macroinvertebrates. *Journal of the North American Benthological Society 14*: 197-202.

Fitzharris, B.B.; Mansergh, G.D.; Soons, J.M. 1992: Basins and lowlands of the South Island. Pp. 406-423 *in:* J.M. Soons; M.J. Selby (editors) *Landforms of New Zealand. Second edition*, Longman Paul, Auckland, New Zealand.

Fletcher, W.J. 1991: A test of the relationship between otolith weight and age for the pilchard *Sardinops neopilchardus*. *Canadian Journal of Fisheries and Aquatic Sciences 48*: 35-38.

Fox, M.G.; Keast, A. 1990: Effects of winterkill on population structure, body size, and prey consumption patterns of pumpkinseed in isolated beaver ponds. *Canadian Journal of Zoology 68*: 2489-2498.

Fox, M.G.; Keast, A. 1991: Effect of overwinter mortality on reproductive life history characteristics of pumpkinseed (*Lepomis gibbosus*) populations. *Canadian Journal of Fisheries and Aquatic Sciences 48*: 1792-1799.

Fox, P.J. 1978: Caddis larvae (Trichoptera) as predators of fish eggs. *Freshwater Biology 8*: 343-345.

Gaggiotti, O.E. 2003: Genetic threats to population persistence. *Annales Zoologici Fennici 40*: 155-168.

Gay, C. 1999: New Zealand freshwater fish. Pp. 18-21 *in:* Proceedings of the Incubation workshop February 1999, New Zealand Conservation Management Group Inc., Lower Hutt, New Zealand.

Gee, J.H. 1980: Respiratory patterns and antipredator responses in the central mudminnow, *Umbra limi*, a continuous, facultative, air-breathing fish. *Canadian Journal of Zoology 58*: 819-827.

Giles, N. 1987: Predation risk and reduced foraging activity in fish: experiments with parasitized and non-parasitized three-spined sticklebacks, *Gasterosteus aculeatus* L. *Journal of Fish Biology 31*: 37-44.

Gillespie, O.A. 1954: *Oxford: the first hundred years*. Oxford Historical Committee, Oxford, Canterbury, New Zealand. 260 p.

Gleeson, D.J.; McCallum, H.I.; Owens, I.P.F. 2000: Differences in initial and acquired resistance to *Ichthyophthirius multifiliis* between populations of rainbowfish. *Journal of Fish Biology 57*: 466-475.

Glova, G.J. 1999: A test for competition between two *Gobiomorphus* species (Teleostei: Eleotridae) in artificial channels. *Ecology of Freshwater Fish 8*: 70-77.

Glova, G.J.; Hulley, G. 1998: Tutaepatu Lagoon devastated by drought. *Water & Atmosphere 6*: 5.

Glova, G.J.; Sagar, P.M.; Näslund, I. 1992: Interaction for food and space between populations of *Galaxias vulgaris* Stokell and juvenile *Salmo trutta* L. in a New Zealand stream. *Journal of Fish Biology 41*: 909-925.

Grainger, N.P.J. 2000: Fire ecology of a pakihi terrace, Westport, South Island, New Zealand. Unpublished MSc thesis, University of Otago, Dunedin, New Zealand. 119 p.

Grand, T.C.; Dill, L.M. 1999: The effect of group size on the foraging behaviour of juvenile coho salmon: reduction of predation risk or increased competition? *Animal Behaviour 58*: 443-451.

GraphPad Software. 1999: Graphpad Prism for Windows. Version 3.0, GraphPad Software, San Diego, United States of America.

Gray, J. 2000: More homes for the Canterbury mudfish. *Forest & Bird 298(1)*: 8.

Green, R.H.; Young, R.C. 1993: Sampling to detect rare species. *Ecological Applications 3*: 351-356.

Grigg, G.C. 1969: The failure of oxygen transport in a fish at low levels of ambient oxygen. *Comparative Biochemistry and Physiology 29*: 1253-1257.

Guma'a, S.A. 1978: The effects of temperature on the development and mortality of eggs of perch, *Perca fluviatilis*. *Freshwater Biology 8*: 221-227.

HACH 1996: DR/2000 *Spectrophotometer Procedures Manual. Edition 3, revision 2*. United States of America. 655 p.

Hamilton, S.; Moller, H. 1995: Can PVA models using computer packages offer useful conservation advice? Sooty shearwaters *Puffinus griseus* in New Zealand as a case study. *Biological Conservation 73*: 107-117.

Hamilton, W.J. 1998: Threat-sensitive nest preferences in male upland bullies, *Gobiomorphus breviceps*. *Ethology 104*: 709-715.

Hamley, J.M.; Howley, T.P. 1985: Factors affecting the variability of trapnet catches. *Canadian Journal of Fisheries and Aquatic Sciences 42*: 1079-1087.

Hand, S.C.; Podrabsky, J.E. 2000: Bioenergetics of diapause and quiescence in aquatic animals. *Thermochimica Acta 349*: 31-42.

Hanski, I.; Ovaskainen, O. 2002: Extinction debt at extinction threshold. *Conservation Biology 16*: 666-673.

Hanski, I.; Simberloff, D. 1997: The metapopulation approach: its history, conceptual domain, and application to conservation. Pp. 5-26 *in:* I. Hanski; M.E. Gilpin (ed.) *Metapopulation Biology, Ecology, Genetics and Evolution*, Academic Press, San Diego, United States of America.

Harraway, S. 2000: Distribution and habitat in the Raukapuka area of the Canterbury mudfish (*Neochanna burrowsius*). Department of Conservation, unpublished report, Raukapuka Area Office. 22 p.

Hartman, G. 1990: Ability of some New Zealand fishes to burrow in gravel. *Freshwater Catch 44*: 15-16.

Harvey, B.C.; Nakamoto, R.J.; White, J.L. 1999: Influence of large woody debris and a bankfull flood on movement of adult resident coastal cutthroat trout (*Oncorhynchus clarki*) during fall and winter. *Canadian Journal of Fisheries and Aquatic Sciences 56*: 2161-2166.

Hay, D.E. 1986: Effects of delayed spawning on viability of eggs and larvae of Pacific herring. *Transactions of the American Fisheries Society 115*: 155-161.

Hayes, J.W. 1989: Comparison between a fine mesh trap net and five other fishing gears for sampling shallow-lake fish communities in New Zealand. *New Zealand Journal of Marine and Freshwater Research 23*: 321-324.

Heath, D.; Roff, D.A. 1987: Test of genetic differentiation in growth of stunted and nonstunted populations of yellow perch and pumpkinseed. *Transactions of the American Fisheries Society 116*: 98-102.

Heimbuch, D.G.; Wilson, H.T.; Weisberg, S.B.; Vølstad, J.H.; Kazyak, P.F. 1997: Estimating fish abundance in stream surveys by using double-pass removal sampling. *Transactions of the American Fisheries Society 126*: 795-803.

Heithaus, M.R.; Laushman, R.H. 1997: Genetic variation and conservation of stream fishes: influence of ecology, life history, and water quality. *Canadian Journal of Fisheries and Aquatic Sciences 54*: 1822-1836.

Hickey, C.W.; Quinn, J.M.; Davies-Colley, R.J. 1989: Effluent characteristics of dairy shed oxidation ponds and their potential impacts on rivers. *New Zealand Journal of Marine and Freshwater Research 23*: 569-584.

Hicks, B.J. 1982: Investigating fish kills. *Freshwater Catch 17*: 10-11.

Hicks, B.J.; Barrier, R.F.G. 1996: Habitat requirements of black mudfish (*Neochanna diversus*) in the Waikato region, North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research 30*: 135-151.

Hicks, B.J.; Reeves, G.H. 1994: Restoration of stream habitat for fish using in-stream structures. Pp. 67-91 *in:* K.J. Collier (editor) *Selected papers from the second day of the New Zealand Limnological Society 1993 annual conference*, Department of Conservation, Wellington, New Zealand.

Hilderbrand, R.H. 2003: The roles of carrying capacity, immigration, and population synchrony on persistence of stream-resident cutthroat trout. *Biological Conservation 110*: 257-266.

Hitchmough, R. (compiler) 2002: New Zealand threat classification system lists. *Threatened Species Occasional Publication 23*. 211 p.

Hixon, M.A.; Pacala, S.W.; Sandin, S.A. 2002: Population regulation: historical context and contemporary challenges of open vs. closed systems. *Ecology 83*: 1490-1508.

Hoffman, G.L. 1973: The effect of certain parasites on North American freshwater fishes. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 18*: 1622-1627.

Holbrook, S.J.; Forrester, G.E.; Schmitt, R.J. 2000: Spatial patterns in abundance of a damselfish reflect availability of suitable habitat. *Oecologia 122*: 109-120.

Holmgren, K.; Appelberg, M. 2000: Size structure of benthic freshwater fish communities in relation to environmental gradients. *Journal of Fish Biology 57*: 1312-1330.

Holopainen, I.J.; Pitkänen, A.K. 1985: Population size and structure of crucian carp (*Carassius carassius* (L.)) in two small, natural ponds in Eastern Finland. *Annales Zoologici Fennici 22*: 397-406.

Holopainen, I.J.; Tonn, W.M.; Paszkowski, C.A. 1997: Tales of two fish: the dichotomous biology of crucian carp (*Carassius carassius* (L.)) in northern Europe. *Annales Zoologici Fennici 34*: 1-22.

Holt, R.D.; Pickering, J. 1985: Infectious disease and species coexistence: a model of Lotka–Volterra form. *The American Naturalist 126*: 196-211.

Hopkins, C.L. 1979: Age-related growth characteristics of *Galaxias fasciatus* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research 13*: 39-46.

Howard-Williams, C.; Davies, J.; Pickmere, S. 1982: The dynamics of growth, the effects of changing area and nitrate uptake by watercress *Nasturtium officinale* R. Br. in a New Zealand stream. *Journal of Applied Ecology 19*: 589-601.

Hoyer, M.V.; Canfield, D.E. 1996: Largemouth bass abundance and aquatic vegetation in Florida lakes: an empirical analysis. *Journal of Aquatic Plant Management 34*: 23-32.

Hudson, H.R.; Harding, J.S. 2004: Drainage management in New Zealand: a review of existing activities and alternative management practices. *Science for Conservation 235*. 39 p.

Hurlbert, S.H. 1990: Spatial distribution of the montane unicorn. *Oikos 58*: 257–271.

Igoe, F.; O'Grady, M.; Byrne, C.; Gargan, P.; Roche, W.; O'Neill, J. 2001: Evidence for the recent extinctions of two Arctic charr *Salvelinus alpinus* (L.) populations in the West of Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems 11*: 77-92.

Jackson, D.A.; Harvey, H.H. 1997: Qualitative and quantitative sampling of lake fish communities. *Canadian Journal of Fisheries and Aquatic Sciences 54*: 2807-2813.

Jacobsen, B.H.; Hansen, M.M.; Loeschcke, V. 2005: Microsatellite DNA analysis of northern pike (*Esox lucius* L.) populations: insights into the genetic structure and demographic history of a genetically depauperate species. *Biological Journal of the Linnean Society 84*: 91-101.

Jansen, W.A.; Mackay, W.C. 1991: Body composition and reproductive investment of stunted yellow perch, *Perca flavescens*. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 24*: 2356-2361.

Jellyman, D.J. 1989: The Canterbury drought and effects on fish. *Freshwater Catch 39*: 22-24.

Jellyman, D.J.; Lambert, P.W. 2004: The how and when of catching glass eels. *Water & Atmosphere 11(4)*: 22-23.

Jenkins, D.G.; Grissom, S.; Miller, K. 2003: Consequences of prairie wetland drainage for crustacean biodiversity and metapopulations. *Conservation Biology 17*: 158-167.

Jobberns, G. 1927: The Canterbury Plains, their origin and structure. Pp. 88-103 *in:* R. Speight; A. Wall; R.M. Laing (editors) *Natural history of Canterbury*, The Philosophical Institute of Canterbury, Christchurch, New Zealand.

Johnson, L. 1994: Long-term experiments on the stability of two fish populations in previously unexploited Arctic lakes. *Canadian Journal of Fisheries and Aquatic Sciences 51*: 209-225.

Johnson, P.N.; Brooke, P.A. 1998: *Wetland plants in New Zealand.* Manaaki Whenua Press, Lincoln, Canterbury, New Zealand. 319 p.

Johnson, P.N.; Gerbeaux, P. 2004: *Wetland types in New Zealand.* Department of Conservation, Wellington, New Zealand. 184 p.

Johnston, C.E. 1999: The relationship of spawning mode to conservation of North American minnows (Cyprinidae). *Environmental Biology of Fishes 55*: 21-30.

Johnston, T.A.; Leggett, W.C. 2002: Maternal and environmental gradients in the egg size of an iteroparous fish. *Ecology 83*: 1777-1791.

Jones, K.E.; Purvis, A.; Gittleman, J.L. 2003: Biological correlates of extinction risk in bats. *The American Naturalist 161*: 601-614.

Jonsson, N.; Jonsson, B. 1999: Trade-off between egg mass and egg number in brown trout. *Journal of Fish Biology 55*: 767-783.

Jowett, I.G. 1998: New Zealand Freshwater Database Assistant Version 5.1. National Institute of Water and Atmospheric Research Ltd, Hamilton, New Zealand.

Jowett, I.G.; Boustead, N.C. 2001: Effects of substrate and sedimentation on the abundance of upland bullies (*Gobiomorphus breviceps*). *New Zealand Journal of Marine and Freshwater Research 35*: 605-613.

Jowett, I.G.; Richardson, J. 1995: Habitat preferences of common, riverine New Zealand native fishes and implications for flow management. *New Zealand Journal of Marine and Freshwater Research 29*: 13-23.

Jowett, I.G.; Richardson, J. 1996: Distribution and abundance of freshwater fish in New Zealand rivers. *New Zealand Journal of Marine and Freshwater Research 30*: 239-255.

Jowett, I.G.; Richardson, J. 2003: Fish communities in New Zealand rivers and their relationship to environmental variables. *New Zealand Journal of Marine and Freshwater Research 37*: 347-366.

Juanes, F. 2003: The allometry of cannibalism in piscivorous fishes. *Canadian Journal of Fisheries and Aquatic Sciences 60*: 594-602.

Karr, J.R. 1982: Population variability and extinction in the avifauna of a tropical land bridge island. *Ecology 63*: 1975-1978.

Keckeis, H.; Bauer-Nemeschkal, E.; Kamler, E. 1996: Effects of reduced oxygen level on the mortality and hatching rate of *Chondrostoma nasus* embryos. *Journal of Fish Biology 49*: 430-440.

Kerfoot, W.C.; Sih, A. 1987: *Predation: direct and indirect impacts on aquatic communities*. University Press of New England, Hanover, New Hampshire, United States of America. 386 p.

Koehn, J.D.; Raadik, T.A. 1991: The Tasmanian mudfish, *Galaxias cleaveri* Scott, 1934, in Victoria. *Proceedings of the Royal Society of Victoria 103*: 77-86.

Koh, L.P.; Sodhi, N.S.; Brook, B.W. 2004: Ecological correlates of extinction proneness in tropical butterflies. *Conservation Biology 18*: 1571-1578.

Kotler, B.P. 1984: Risk of predation and the structure of desert rodent communities. *Ecology 65*: 689-700.

Kotliar, N.B.; Wiens, J.A. 1990: Multiple scales of patchiness and patch structure: a hierarchical framework for the study of heterogeneity. *Oikos 59*: 253-260.

Koumoundouros, G.; Divanach, P.; Kentouri, M. 2001: The effect of rearing conditions on development of saddleback syndrome and caudal fin deformities in (*Dentex dentex* (L.)). *Aquaculture 200*: 285-304.

Koya, Y.; Kamiya, E. 2000: Environmental regulation of annual reproductive cycle in the mosquitofish, *Gambusia affinis*. *Journal of Experimental Zoology 286*: 204-211.

Kramer, D.L.; Graham, J.B. 1976: Synchronous air breathing, a social component of respiration in fishes. *Copeia 1976*: 689-697.

Krause, J.; Loader, S.P.; McDermott, J.; Ruxton, G.D. 1998: Refuge use by fish as a function of body length-related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London Part B 265*: 2373-2379.

Krebs, C.J. 1994: *Ecology: the experimental analysis of distribution and abundance. Fourth edition*. HarperCollins College Publishers, New York, United States of America. 801 p.

Krebs, C.J. 1998: *Ecological methodology*. Benjamin/Cummings, Menlo Park, California, United States of America. 620 p.

Kume, G.; Yamaguchi, A.; Aoki, I. 2002: Dummy egg production by female cardinalfish to deceive cannibalistic males: oogenesis without vitellogenesis. *Environmental Biology of Fishes 65*: 469-472.

Kusabs, I.A.; Swales, S. 1991: Diet and food resource partitioning in koaro, *Galaxias brevipinnis* (Günther), and juvenile rainbow trout, *Oncorhynchus mykiss* (Richardson), in two Taupo streams. *New Zealand Journal of Marine and Freshwater Research 25*: 317-325.

Lake, P.S. 2000: Disturbance, patchiness, and diversity in streams. *Journal of the North American Benthological Society 19*: 573-592.

Laland, K.N.; Williams, K. 1997: Shoaling generates social learning of foraging information in guppies. *Animal Behaviour 53*: 1161-1169.

Lam, T.J.; Nagahama, Y.; Chan, K.; Hoar, W.S. 1978: Overripe eggs and postovulatory corpora lutea in the threespine stickleback, *Gasterosteus aculeatus* L., form *trachurus*. *Canadian Journal of Zoology 56*: 2029-2036.

Latham, K.E.; Just, J.J. 1989: Oxygen availability provides a signal for hatching in the rainbow trout (*Salmo gairdneri*) embryo. *Canadian Journal of Fisheries and Aquatic Sciences 46*: 55-58.

Laughlin, D.R.; Werner, E.E. 1980: Resource partitioning in two coexisting sunfish: pumpkinseed (*Lepomis gibbosus*) and northern longear sunfish (*Lepomis megalotis peltastes*). *Canadian Journal of Fisheries and Aquatic Sciences 37*: 1411-1420.

Le Cren, E.D. 1951: The weight – length relationship and seasonal cycle in gonad weight and the condition in perch (*Perca fluviatilis*). *Journal of Animal Ecology 20*: 201-219.

Le Cren, E.D. 1965: Some factors regulating the size of populations of freshwater fish Mitteilungen. *Internationale Vereinigung für Theoretische und Angewandte Limnologie 13*: 88-105.

LeCren, E.D. 1990: Rare fishes and their conservation: a brief introduction to the symposium. *Journal of Fish Biology 37 (Supplement A)*: 1-3.

Lehmann, A.; Lachavanne, J.-B. 1999: Changes in the water quality of Lake Geneva indicated by submerged macrophytes. *Freshwater Biology 42*: 457-466.

Lemly, A.D. 1994: Irrigated agriculture and freshwater wetlands: A struggle for coexistence in the western United States. *Wetlands Ecology and Management 3*: 3-15.

Levels, P.J.; Gubbels, R.E.; Denuce, J.M. 1986: Oxygen consumption during embryonic development of the annual fish *Nothobranchius korthausae* with special reference to diapause. *Comparative Biochemistry and Physiology A 84*: 767-770.

Lima, S.L.; Dill, L.M. 1990: Behavioural decisions made under risk of predation: a review and prospectus. *Canadian Journal of Zoology 68*: 619-640.

Ling, N.; Barrier, R.F.G.; O'Brien, L.K.; Miller, R. no date: Methodology to survey and monitor NZ mudfish species. Unpublished report to Department of Conservation, Wellington, New Zealand.

Lobón-Cerviá, J.; Utrilla, C.G.; Rincón, P.A.; Amezcua, F. 1997: Environmentally induced spatio-temporal variations in the fecundity of brown trout *Salmo trutta* L.: trade-offs between egg size and number. *Freshwater Biology 38*: 277-288.

Lopukhina, A.M.; Strelkov, Y.A.; Chernyshova, N.B.; Yunchis, O.N. 1973: The effect of parasites on abundance of young fish in natural water bodies. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 18*: 1705-1712.

Ludwig, D. 1999: Is it meaningful to estimate a probability of extinction? *Ecology 80*: 298-310.

Magoulick, D.D.; Kobza, R.M. 2003: The role of refugia for fishes during drought: a review and synthesis. *Freshwater Biology 48*: 1186-1198.

Main, M.R.; Meredith, A.S. 1999: Fishery, water quality, recreational, and wildlife impacts of the 1997-98 and 1998-99 droughts in Canterbury. Technical Report: Environmental Monitoring Group U99/61, Environment Canterbury, Christchurch, New Zealand. 22 p.

Maitland, P.S. 1995: The conservation of freshwater fish: past and present experience. *Biological Conservation 72*: 259-270.

Mangor-Jensen, A.; Waiwood, K.G.; Peterson, R.H. 1993: Water balance in eggs of striped bass (*Morone saxatilis*). *Journal of Fish Biology 43*: 345-353.

Margenau, T.L.; Rasmussen, P.W.; Kampa, J.M. 1998: Factors affecting growth of northern pike in small Northern Wisconsin lakes. *North American Journal of Fisheries Management 18*: 625-639.

May, J.T.; Brown, L.R. 2002: Fish communities of the Sacramento River Basin: implications for conservation of native fishes in the Central Valley, California. *Environmental Biology of Fishes 63*: 373-388.

McClay, C.L. 1976: The effect of pH on the population growth of three species of duckweed: *Spirodela oligorrhiza, Lemna minor* and *Wolffia arrhiza*. *Freshwater Biology 6*: 125-136.

McCoy, E.D.; Bell, S.S. 1991: Habitat structure: the evolution and diversification of a complex topic. Pp. 3-21 *in:* S.S. Bell; E.D. McCoy; H.R. Mushinsky (editors) *Habitat structure: the physical arrangement of objects in space*, Chapman & Hall, Suffolk, United Kingdom.

McDowall, R.M. 1968: Interactions of the native and alien faunas of New Zealand and the problem of fish introductions. *Transactions of the American Fisheries Society 97*: 1-11.

McDowall, R.M. 1969: Lunar rhythms in aquatic animals: a general review. *Tuatara 17*: 133-144.

McDowall, R.M. 1970: The galaxiid fishes of New Zealand. *Bulletin of the Museum of Comparative Zoology, Harvard University 139*: 341-432.

McDowall, R.M. 1982: Fish in swamps. *Freshwater Catch 15*: 9-11.

McDowall, R.M. 1988: Moon affects fish behaviour. *Freshwater Catch 38*: 18.

McDowall, R.M. 1990: *New Zealand freshwater fishes: a natural history and guide*. Heinemann Reed and MAF Publishing Group, Auckland, New Zealand. 553 p.

McDowall, R.M. 1997a: Affinities, generic classification and biogeography of the Australian and New Zealand mudfishes (Salmoniformes: Galaxiidae). *Records of the Australian Museum 49*: 121-137.

McDowall, R.M. 1997b: An accessory lateral line in some New Zealand and Australian galaxiids (Teleostei: Galaxiidae). *Ecology of Freshwater Fish 6*: 217-224.

McDowall, R.M. 1998: Once were wetlands. *Fish & Game 20*: 32-39.

McDowall, R.M.; Eldon, G.A. 1997: Reproductive cycling and fecundity estimation in the upland bully. *Journal of Fish Biology 51*: 164-179.

McDowall, R.M.; Richardson, J. 1983: New Zealand freshwater fish survey: a guide to input and output. *New Zealand Ministry of Agriculture and Fisheries, Fisheries Information Leaflet 12*.15 p.

McIntosh, A.R.; Townsend, C.R.; Crowl, T.A. 1992: Competition for space between introduced brown trout (*Salmo trutta* L.) and common river galaxias (*Galaxias vulgaris* Stokell) in a New Zealand stream. *Journal of Fish Biology 41*: 63-81.

McPhail, J.D. 1999: A fish out of water: observations on the ability of black mudfish, *Neochanna diversus,* to withstand hypoxic water and drought. *New Zealand Journal of Marine and Freshwater Research 33*: 417-424.

Medeiros, E.S.F.; Maltchik, L. 2000: Influence of hydrological disturbances on the reproduction of a fish community in an intermittent stream from the Brazilian semiarid

region. *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 27*: 906-911.

Meredith, A.S. 1981: Respiration and aestivation of the Canterbury mudfish *Neochanna burrowsius* (Galaxiidae). Unpublished BSc (Honours) project, University of Canterbury, Christchurch, New Zealand. 73 p.

Meredith, A.S. 1985: Metabolism and cutaneous exchange in an amphibious fish *Neochanna burrowsius* (Phillipps). Unpublished PhD thesis, University of Canterbury, Christchurch, New Zealand. 242 p.

Meredith, A.S.; Davie, P.S.; Forster, M.E. 1982: Oxygen uptake by the skin of the Canterbury mudfish, *Neochanna burrowsius*. *New Zealand Journal of Zoology 9*: 387-390.

Mesa, M.G.; Schreck, C.B. 1989: Electrofishing mark-recapture and depletion methodologies evoke behavioural and physiological changes in cutthroat trout. *Transactions of the American Fisheries Society 118*: 644-658.

MfE (Ministry for the Environment) 2001: Managing waterways on farms: a guide to sustainable water and riparian management in rural New Zealand. Ministry for the Environment, Wellington, New Zealand. 209 p.

Microsoft Corporation 2002: Excel Version 10. Redmond, Washington, United States of America.

Mitchell, C.P.; Penlington, B.P. 1982: Spawning of *Galaxias fasciatus* Gray (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research 16*: 131-133.

Mittelbach, G.G.; Chesson, P.L. 1987: Predation risk: indirect effects on fish populations. Pp. 315-332 *in:* W.C. Kerfoot; A. Sih (editors) *Predation: direct and indirect impacts on aquatic communities*, University Press of New England, Hanover, New Hampshire, United States of America.

Mommsen, T.P. 2001: Paradigms of growth in fish. *Comparative Biochemistry and Physiology Part B 129*: 207-219.

Moodie, G.E.E.; Loadman, N.L.; Wiegand, M.D.; Mathias, J.A. 1989: Influence of egg characteristics on survival, growth and feeding in larval walleye (*Stizostedion vitreum*). *Canadian Journal of Fisheries and Aquatic Sciences 46*: 516-521.

Moore, S.J.; Allibone, R.M.; Townsend, C.R. 1999: Spawning site selection by two galaxiid fishes, *Galaxias anomalus* and *G. depressiceps*, in tributaries of the Taieri River, South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research 33*: 129-139.

Morgan, M.; Bidwell, V.; Bright, J.; McIndoe, I.; Robb, C. 2002: Canterbury strategic water study. pp. 258, Lincoln Environmental, Lincoln, Canterbury, New Zealand.

Morita, K.; Yamamoto, S.; Takashima, Y.; Matsuishi, T.; Kanno, Y.; Nishimura, K. 1999: Effect of maternal growth history on egg number and size in wild white-spotted char (*Salvelinus leucomaenis*). *Canadian Journal of Fisheries and Aquatic Sciences 56*: 1585-1589.

Moxley, D. J.; Langford; F. H. 1982: Beneficial effects of hydrilla on two eutrophic lakes in central Florida. *Proceedings of the Annual Conference Southeastern Association of Game and Fish Agencies 36*: 280-286.

Moyle, P.B. 1995: Conservation of native freshwater fishes in the Mediterranean type climate of California, USA: a review. *Biological Conservation 72*: 271-280.

Mugiya, Y.; Uchimura, T. 1989: Otolith resorption induced by anaerobic stress in the goldfish, *Carassius auratus*. *Journal of Fish Biology 35*: 813-818.

Mullan, B.; Porteous, A.; Wratt, D.; Hollis, M. 2005: Changes in drought risk with climate change. NIWA Client Report: WLG2005-23. National Institute of Water and Atmospheric Research, Wellington, New Zealand. 58 p.

Nakai, K.; Yanagisawa, Y.; Sato, T.; Nimura, Y.; Gashagaza, M.M. 1990: Lunar synchronization of spawning in cichlid fishes of the tribe Lamprologini in Lake Tanganyika. *Journal of Fish Biology 37*: 589-598.

Nielsen, L.A.; Johnson, D.L. 1983: *Fisheries techniques*. American Fisheries Society, Bethesda, Maryland, United States of America. 468 p.

NIWA (National Institute of Water and Atmospheric Research). 2004: Freshwater Information New Zealand. Online GIS mapping application. (http://finz.niwa.co.nz).

NIWA (National Institute of Water and Atmospheric Research). No date: Electric fishing machine operator's training course. National Institute of Water and Atmospheric Research Ltd, Christchurch, New Zealand. 42 p.

Nugent, G. 1999: LunarPhase Version 2.50. Dublin, Ireland.

Oborny, B.; Meszéna, G.; Szabó, G. 2005: Dynamics of populations on the verge of extinction. *Oikos 109*: 291-296.

O'Brien, L.K. 2000: Fish community dynamics in Canterbury spring systems. Pp. 6-7 *in* The changing face of freshwater fisheries in New Zealand. A symposium to honour the contribution of Bob McDowall to New Zealand's freshwater science, National Institute of Water and Atmospheric Research, Christchurch.

O'Brien, L.K. 2001: Importance of the Hororata spring wetland to the conservation of Canterbury mudfish. Report to the Department of Conservation, Christchurch. 10 p.

O'Brien, L.K.; Dunn, N.R. 2005: Captive management of mudfish *Neochanna* (Teleostei: Galaxiidae) spp. *DOC Research & Development Series 205*. 29 p.

O'Brien, L.K.; Dunn, N.R. in press: Mudfish: a review of literature regarding *Neochanna* (Teleostei: Galaxiidae). Department of Conservation, Wellington.

Omori K. 1995: The adaptive significance of a lunar or semi-lunar reproductive cycle in marine animals. *Ecological Modelling 82*: 41–49.

Oppen-Berntsen, D.O.; Helvik, J.V.; Walther, B.T. 1990: The major structural proteins of cod (*Gadus morhua*) eggshells and protein crosslinking during teleost egg hardening. *Developmental Biology 137*: 258-265.

Pannella, G. 1974: Otolith growth patterns: an aid in age determination in temperate and tropical fishes. Pp. 28-39 *in:* T.B. Bagenal (editor) *The ageing of fish*, Unwin Brothers Ltd., Surrey, England.

Parker, H.H.; Noonburg, E.G.; Nisbet, R.M. 2001: Models of alternative life-history strategies, population structure and potential speciation in salmonid fish stocks. *Journal of Animal Ecology 70*: 260-272.

Partridge, T.R. 1982: Types of wetland and their botanical diversity. *Freshwater Catch 15*: 5-7.

Patten, B.G. 1968: Abnormal freshwater fishes in Washington streams. *Copeia 2*:399–401. (Original not seen).

Perrie, A. 2004: The life history and ecophysiology of black mudfish (*N. diversus*). Unpublished MSc thesis, University of Waikato, Hamilton, New Zealand. 78 p.

Persat, H. 1991: The efficiency of electric fishing in fish population studies: an experiment on grayling (*Thymallus thymallus*). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 24*: 2432-2436.

Persson, L.; Byström, P.; Wahlström, E. 2000: Cannibalism and competition in Eurasian perch: population dynamics of an ontogenetic omnivore. *Ecology 81*: 1058-1071.

Persson, L.; Greenberg, L.A. 1990: Interspecific and intraspecific size class competition affecting resource use and growth of perch, *Perca fluviatilis*. *Oikos 59*: 97-106.

Pettersson, L.B.; Brönmark, C. 1997: Density-dependent costs of an inducible morphological defence in crucian carp. *Ecology 78*: 1805-1815.

Phillipps, W.J. 1926: New or rare fishes of New Zealand. *Transactions and Proceedings of the New Zealand Institute 56*: 529-537.

Piironen, J.; Holopainen, I.J. 1988: Length structure and reproductive potential of crucian carp (*Carassius carassius* (L.)) populations in some small forest ponds. *Annales Zoologici Fennici 25*: 203-208.

Pister, E.P. 1999: Professional obligations in the conservation of fishes. *Environmental Biology of Fishes 55*: 13-20.

Podrabsky, J.E.; Hand, S.C. 1999: The bioenergetics of embryonic diapause in an annual killifish, *Austrofundulus limnaeus*. *The Journal of Experimental Biology 202*: 2567-2580.

Poff, N.L.; Ward, J.V. 1989: Implications of stream flow variability and predictability for lotic community structure: a regional analysis of streamflow patterns. *Canadian Journal of Fisheries and Aquatic Sciences 46*: 1805-1818.

Portt, C.B.; Balon, E.K.; Noakes, D.L.G. 1986: Biomass and production of fishes in natural and channelized streams. *Canadian Journal of Fisheries and Aquatic Sciences 43*: 1926-1934.

Post, J.R.; Parkinson, E.A.; Johnston, N.T. 1999: Density-dependent processes in structured fish populations: interaction strengths in whole-lake experiments. *Ecological Monographs 69*: 155-175.

Poulin, R. 1993: Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. *Oecologia 96*: 431-438.

Power, M.E.; Stout, R.J.; Cushing, C.E.; Harper, P.P.; Hauer, F.R.; Matthews, W.J.; Moyle, P.B.; Statzner, B.; Wais de Badgen, I.R. 1988: Biotic and abiotic controls in river and stream communities. *Journal of the North American Benthological Society 7*: 456-479.

Power, M.; Dempson, J.B.; Power, G.; Reist, J.D. 2000: Environmental influences on an exploited anadromous Arctic charr stock in Labrador. *Journal of Fish Biology 57*: 82-98.

Raimondo, S.; Liebhold, A.M.; Strazanac, S.; Butler, L. 2004: Population synchrony within and among Lepidoptera species in relation to weather, phylogeny, and larval phenology. *Ecological Entomology 29*: 96-105.

Readers Digest (editors) 1985: *Complete book of New Zealand birds*. Readers Digest, Sydney, Australia. 319 p.

Resetarits, W.J. 1997: Interspecific competition and qualitative competitive asymmetry between two benthic stream fish. *Oikos 78*: 429-439.

Resh, V.H.; Brown, A.V.; Covich, A.P.; Gurtz, M.E.; Li, H.W.; Minshall, G.W.; Reice, S.R.; Sheldon, A.L.; Wallace, J.B.; Wissmar, R.C. 1988: The role of disturbance in stream ecology. *Journal of the North American Benthological Society 7*: 433-455.

Reznick, D.N.; Lindbeck, E.; Bryga, H. 1989: Slower growth results in larger otoliths: an experimental test with guppies (*Poecilia reticulata*). *Canadian Journal of Fisheries and Aquatic Sciences 46*: 108-112.

Rideout, R.M.; Burton, P.M.; Rose, G.A. 2000: Observations on mass atresia and skipped spawning in northern Atlantic cod, from Smith Sound, Newfoundland. *Journal of Fish Biology 57*: 1429-1440.

Riget, F.; Jeppesen, E.; Landkildehus, F.; Lauridsen, T.L.; Geertz-Hansen, P.; Christoffersen, K.; Sparholt, H. 2000: Landlocked Arctic charr (*Salvelinus alpinus*) population structure and lake morphometry in Greenland – is there a connection? *Polar Biology 23*: 550-558.

Riis, T.; Biggs, B.J.F. 2001: Distribution of macrophytes in New Zealand streams and lakes in relation to disturbance frequency and resource supply—a synthesis and conceptual model. *New Zealand Journal of Marine and Freshwater Research 35*: 255-267.

Riis, T.; Biggs, B.J.F.; Flanagan, M. 2003: Seasonal changes in macrophyte biomass in South Island lowland streams, New Zealand. *New Zealand Journal of Marine and Freshwater Research 37*: 381-388.

Rikardsen, A.H.; Elliott, J.M. 2000: Variations in juvenile growth, energy allocation and life-history strategies of two populations of Arctic charr in North Norway. *Journal of Fish Biology 56*: 328-346.

Rodd, F.H.; Reznick, D.N. 1997: Variation in the demography of guppy populations: the importance of predation and life histories. *Ecology 78*: 405-418.

Rodríguez, M.A. 2002: Restricted movement in stream fish: the paradigm is incomplete, not lost. *Ecology 83*: 1-13.

Rose, G.A.; Kulka, D.W. 1999: Hyperaggregation of fish and fisheries: how catch-per-unit-effort increased as the northern cod (*Gadus morhua*) declined. *Canadian Journal of Fisheries and Aquatic Sciences 56 (Supplement 1)*: 118-127.

Ryan, P.M.; Kerekes, J.J. 1989: Correction of relative fish abundance estimates from catch data for variable fishing intensity during lake surveys. *Canadian Journal of Fisheries and Aquatic Sciences 46*: 1022-1025.

Sadler, J.; Pankhurst, P.M.; King, H.R. 2001: High prevalence of skeletal deformity and reduced gill surface area in triploid Atlantic salmon (*Salmo salar* (L.)). *Aquaculture 198*: 369-386.

Sagar, P.M. 1985: Effects of drought on salmonids in an irrigation scheme. *Freshwater Catch 28*: 23-24.

Sagar, P.M. 1986: The effects of floods on the invertebrate fauna of a large, unstable braided river. *New Zealand Journal of Marine and Freshwater Research 20*: 37-46.

Sagar, P.M.; Glova, G.J. 1994: Food partitioning by small fish in a coastal New Zealand stream. *New Zealand Journal of Marine and Freshwater Research 28*: 429-436.

Sagar, P.M.; Jellyman, D.J. 1985: Regional modifications to waterways. Part VI – South Canterbury. *Freshwater Catch 27*: 19-24.

Sand-Jensen, K.; Pedersen, O. 1999: Velocity gradients and turbulence around macrophyte stands in streams. *Freshwater Biology 42*: 315-328.

Schiemer, F.; Keckeis, H.; Kamlar, E. 2003: The early life history stages of riverine fish: ecophysiological and environmental bottlenecks. *Comparative Biochemistry and Physiology Part A 133*: 439-449.

Schubert, G. 1987: *Fish diseases: a complete introduction.* T. F. H. Publications, Inc., Neptune City, New Jersey, United States of America. 125 p.

Schwanck, E. 1987: Lunar periodicity in the spawning of *Tilapia mariae* in the Ethiop River, Nigeria. *Journal of Fish Biology 30*: 533-537.

Scrimgeour, G.J.; Winterbourn, M.J. 1987: Diet, food resource partioning and feeding periodicity of two riffle-dwelling fish species in a New Zealand river. *Journal of Fish Biology 31*: 309-324.

Secor, D.H.; Dean, J.M. 1989: Somatic growth effects on the otolith – fish size relationships in young pond-reared striped bass, *Morone saxatilis*. *Canadian Journal of Fisheries and Aquatic Sciences 46*: 113-121.

Seddon, B. 1972: Aquatic macrophytes as limnological indicators. *Freshwater Biology 2*: 107-130.

Shea, K.; Mangel, M. 2001: Detection of population trends in threatened coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences 58*: 375-385.

Siefert, R.E.; Spoor, W.A.; Syrett, R.F. 1973: Effects of reduced oxygen concentrations on northern pike (*Esox lucius*) embryos and larvae. *Journal of the Fisheries Research Board of Canada 30*: 849-852.

Sih, A. 1992: Prey uncertainty and the balancing of antipredator and feeding needs. *The American Naturalist 139*: 1052-1069.

Skrzynski, W. 1968: The Canterbury mudfish, *Galaxias burrowsius* Phillipps, a vanishing species. *New Zealand Journal of Marine and Freshwater Research 2*: 688-697.

Smale, M.A.; Rabeni, C.F. 1995: Influences of hypoxia and hyperthermia on fish species composition in headwater streams. *Transactions of the American Fisheries Society 124*: 711-725.

Snieszko, S.F. 1974: The effects of environmental stress on outbreaks of infectious disease of fishes. *Journal of Fish Biology 6*: 197-208.

Söderbäck, B. 1994: Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia 100*: 229-235.

Spranza, J.J.; Stanley, E.H. 2000: Condition, growth, and reproductive styles of fishes exposed to different environmental regimes in a prairie drainage. *Environmental Biology of Fishes 59*: 99-109.

Staples, D.J. 1975: Production biology of the upland bully *Philypnodon breviceps* Stokell in a small New Zealand lake. I. Life history, food, feeding and activity rhythms. *Journal of Fish Biology 7*: 1-24.

Stark, J.D. 1985: A macroinvertebrate community index of water quality for stony streams. National Water and Soil Conservation Authority, Wellington, New Zealand. 53 p.

Statsoft Inc. 2001: STATISTICA for Windows. Version 6.0, Statsoft Inc., Tulsa, Oklahoma, United States of America.

Stokell, G. 1938: A new species of the genus *Galaxias*, with a note on the second occurrence of *Galaxias burrowsii* Phillipps. *Records of the Canterbury Museum IV*: 203-208.

Stokell, G. 1940: A new species of *Galaxias*. *Transactions of the Royal Society of New Zealand 69*: 422-424.

Stokell, G. 1949: The systematic arrangement of the New Zealand Galaxiidae. Part II. Specific classification. *Transactions of the Royal Society of New Zealand 77*: 472-496.

Studholme, B. 2002: Koi carp and Gambusia. The South Island pest fish survey 2001-2002. Nelson/Marlborough Conservancy, Department of Conservation, Nelson, New Zealand. 37 p.

Swales, S. 1991: Threats and conservation of native fish. *Freshwater Catch 45*: 19-21.

Swanson, C.; Mager, R.C.; Doroshov, S.I.; Cech, J.C. 1996: Use of salts, anaesthetics, and polymers to minimize handling and transport mortality in delta smelt. *Transactions of the American Fisheries Society 125*: 326-329.

Taylor, M.H. 1984: Lunar synchronization of fish reproduction. *Transactions of the American Fisheries Society 113*: 484-493.

Taylor, M.J.; O'Brien, L.K. 2000: Habitat enhancement and ecology of Canterbury mudfish in the Mounsey's Wetland. National Institute of Water and Atmospheric Research, Christchurch, New Zealand. 30 p.

Te Rünanga o Ngäi Tahu; Her Majesty the Queen 1997: Ngäi Tahu Deed of Settlement. Office of Treaty Settlements, Wellington, New Zealand. 1790 p.

Tonn, W.M.; Paszkowski, C.A. 1986: Size-limited predation, winterkill, and the organization of *Umbra-Perca* fish assemblages. *Canadian Journal of Fisheries and Aquatic Sciences 43*: 194-202.

Town, J. 1981: Black mudfish in Whangamarino. *Freshwater Catch 12*: 9.

Townsend, C.R. 1989: The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society 8*: 36-50.

Valdimarsson, S.K.; Skúlason, S.; Snorrason, S.S. 2002: The relationship between egg size and the rate of early development in Arctic charr, *Salvelinus alpinus*. *Environmental Biology of Fishes 65*: 463-468.

van Duijn, C. 1973: *Diseases of fishes*. Iliffe Books, London, England. 372 p.

Van Weerd, J.H.; Komen, J. 1998: The effects of chronic stress on growth in fish: a critical appraisal. *Comparative Biochemistry and Physiology Part A 120*: 107-112.

Vik, J.O.; Borgstrøm, R.; Skaala, Ø. 2001: Cannibalism governing mortality of juvenile brown trout, *Salmo trutta*, in a regulated stream. *Regulated Rivers: Research and Management 17*: 583-594.

Vila-Gispert, A.; Moreno-Amich, R. 2001: Mass-length relationship of Mediterranean barbel as an indictor of environmental status in South-west European stream ecosystems. *Journal of Fish Biology 59*: 824-832.

Stokell, G. 1940: A new species of *Galaxias*. *Transactions of the Royal Society of New Zealand 69*: 422-424.

Stokell, G. 1949: The systematic arrangement of the New Zealand Galaxiidae. Part II. Specific classification. *Transactions of the Royal Society of New Zealand 77*: 472-496.

Studholme, B. 2002: Koi carp and Gambusia. The South Island pest fish survey 2001-2002. Nelson/Marlborough Conservancy, Department of Conservation, Nelson, New Zealand. 37 p.

Swales, S. 1991: Threats and conservation of native fish. *Freshwater Catch 45*: 19-21.

Swanson, C.; Mager, R.C.; Doroshov, S.I.; Cech, J.C. 1996: Use of salts, anaesthetics, and polymers to minimize handling and transport mortality in delta smelt. *Transactions of the American Fisheries Society 125*: 326-329.

Taylor, M.H. 1984: Lunar synchronization of fish reproduction. *Transactions of the American Fisheries Society 113*: 484-493.

Taylor, M.J.; O'Brien, L.K. 2000: Habitat enhancement and ecology of Canterbury mudfish in the Mounsey's Wetland. National Institute of Water and Atmospheric Research, Christchurch, New Zealand. 30 p.

Te Rünanga o Ngäi Tahu; Her Majesty the Queen 1997: Ngäi Tahu Deed of Settlement. Office of Treaty Settlements, Wellington, New Zealand. 1790 p.

Tonn, W.M.; Paszkowski, C.A. 1986: Size-limited predation, winterkill, and the organization of *Umbra-Perca* fish assemblages. *Canadian Journal of Fisheries and Aquatic Sciences 43*: 194-202.

Town, J. 1981: Black mudfish in Whangamarino. *Freshwater Catch 12*: 9.

Townsend, C.R. 1989: The patch dynamics concept of stream community ecology. *Journal of the North American Benthological Society 8*: 36-50.

Valdimarsson, S.K.; Skúlason, S.; Snorrason, S.S. 2002: The relationship between egg size and the rate of early development in Arctic charr, *Salvelinus alpinus*. *Environmental Biology of Fishes 65*: 463-468.

van Duijn, C. 1973: *Diseases of fishes*. Iliffe Books, London, England. 372 p.

Van Weerd, J.H.; Komen, J. 1998: The effects of chronic stress on growth in fish: a critical appraisal. *Comparative Biochemistry and Physiology Part A 120*: 107-112.

Vik, J.O.; Borgstrøm, R.; Skaala, Ø. 2001: Cannibalism governing mortality of juvenile brown trout, *Salmo trutta*, in a regulated stream. *Regulated Rivers: Research and Management 17*: 583-594.

Vila-Gispert, A.; Moreno-Amich, R. 2001: Mass-length relationship of Mediterranean barbel as an indictor of environmental status in South-west European stream ecosystems. *Journal of Fish Biology 59*: 824-832.

Viljanen, M.; Koho, J. 1991: The effects of egg size and incubation conditions on life history of vendace (*Coregonus albula* L.). *Verhandlungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie 24*: 2418-2423.

Walsh, W.A.; Swanson, C.; Lee, C.L.; Banno, J.E.; Eda, H. 1989: Oxygen consumption by eggs and larvae of striped mullet, *Mugil cephalus,* in relation to development, salinity and temperature. *Journal of Fish Biology 35*: 347-358.

Wang, S.; Hard, J.J.; Utter, F. 2002: Salmonid inbreeding: a review. *Reviews in Fish Biology and Fisheries 11*: 301-319.

Warren, M.L.; Burr, B.M.; Walsh, S.J.; Bart, H.L.; Cashner, R.C.; Etnier, D.A.; Freeman, B.J.; Kuhajda, B.R.; Mayden, R.L.; Robison, H.W.; Ross, S.T.; Starnes, W.C. 2000: Diversity, distribution, and conservation status of the native freshwater fishes of the Southern United States. *Fisheries 25*: 7-29.

Weatherley, A.H.; Gill, H.S. 1987: *The biology of fish growth.* Academic Press, London, England. 443 p.

Weber, E.D.; Fausch, K.D. 2003: Interactions between hatchery and wild salmonids in streams: differences in biology and evidence for competition. *Canadian Journal of Fisheries and Aquatic Sciences 60*: 1018-1036.

Wells, R.M.G.; Forster, M.E.; Meredith, A.S. 1984: Blood oxygen affinity in the amphibious fish *Neochanna burrowsius* (Galaxiidae: Salmoniformes). *Physiological Zoology 57*: 261-265.

Werner, E.E.; Hall, D.J. 1977: Competition and habitat shift in two sunfishes (Centrarchidae). *Ecology 58*: 869-876.

Werner, E.E.; Hall, D.J. 1979: Foraging efficiency and habitat switching in competiting sunfishes. *Ecology 60*: 256-264.

Wiegand, M.D.; Hataley, J.M.; Kitchen, C.L.; Buchanan, L.G. 1989: Induction of developmental abnormalities in larval goldfish, *Carassius auratus* L., under cool incubation conditions. *Journal of Fish Biology 35*: 85-95.

Wiens, J.A. 1989: Spatial scaling in ecology. *Functional Ecology 3*: 385-397.

Wilcock, R.J.; McBride, G.B.; Nagels, J.W.; Northcott, G.L. 1995: Water quality in a polluted lowland stream with chronically depressed dissolved oxygen: causes and effects. *New Zealand Journal of Marine and Freshwater Research 29*: 277-288.

Wilcock, R.J.; Nagels, J.W.; McBride, G.B.; Collier, K.J.; Wilson, B.T.; Huser, B.A. 1998: Characterisation of lowland streams using a single-station diurnal curve analysis model with continuous monitoring data for dissolved oxygen and temperature. *New Zealand Journal of Marine and Freshwater Research 32*: 67-79.

Wilson, E.W.S. 1982: Wetlands – their agricultural and horticultural importance. *Freshwater Catch 15*: 8-9.

Winterbourn, M.J.; Gregson, K.L.D.; Dolphin, C.H. 2000: Guide to the aquatic insects of New Zealand. *Bulletin of the Entomological Society of New Zealand 13*: 102.

Winterbourn, M.J.; Rounick, J.S.; Cowie, B. 1981: Are New Zealand stream ecosystems really different? *New Zealand Journal of Marine and Freshwater Research 15*: 321-328.

Wolf, N.G.; Kramer, D.L. 1987: Use of cover and the need to breathe: the effects of hypoxia on vulnerability of dwarf gouramis to predatory snakeheads. *Oecologia 73*: 127-132.

Wurtsbaugh, W.A.; Tapia, R.A. 1988: Mass mortality of fishes in Lake Titicaca (Peru-Bolivia) associated with the protozoan parasite *Ichthyophthirius multifiliis*. *Transactions of the American Fisheries Society 117*: 213-217.

Ylikarjula, J.; Heino, M.; Dieckmann, U. 1999: Ecology and adaptation of stunted growth in fish. International Institute for Applied Systems Analysis, Laxenburg, Austria. 18 p.

Young, D. 1996: In search of mudfish. *Forest & Bird 279(1)*: 14-17.

Appendix 1. Development stages of eggs.

Spawned *N. burrowsius* eggs were categorised into three macroscopically identifiable developmental stages. These stages were then described and checked microscopically. Stage I eggs lacked any pigment. Stage II eggs exhibited retinal pigmentation, but lacked full retinal development, and embryos had no body pigmentation. Whereas in stage III eggs retinal development and body melanophore pigmentation was complete. Furthermore, stage III embryos had a distinct yellow coloration. To determine the progression of development through the three stages identified, three replicates, each of ten newly spawned eggs were held at 15 °C and examined microscopically daily (Figure A.1).

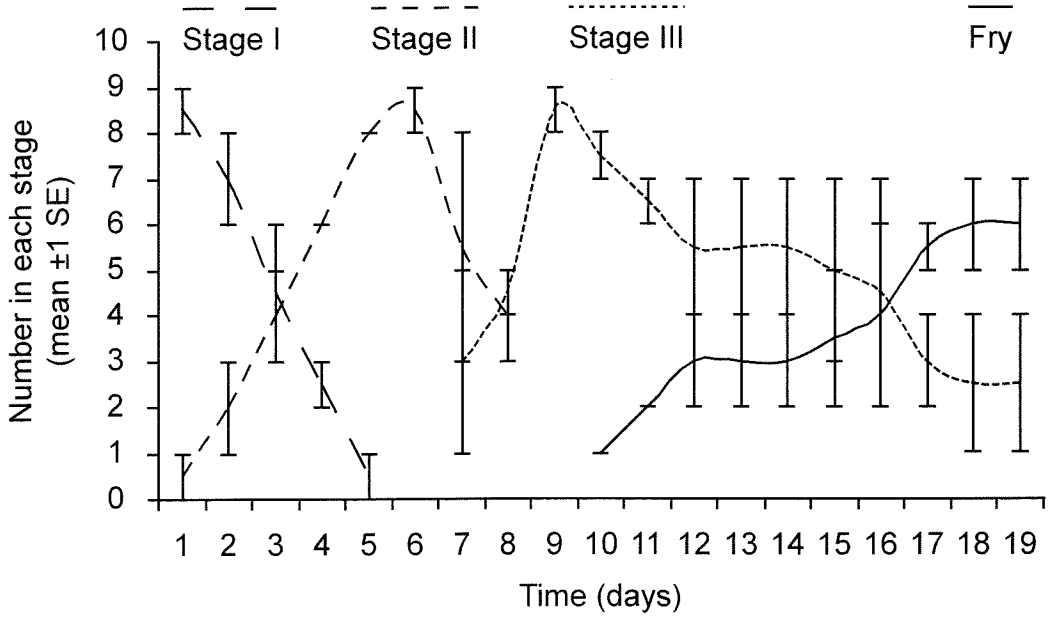

Figure A.1. Daily number of eggs in the three stages of development described in this thesis, when held at 15 °C. Error bars indicate plus or minus one standard error.